Mammalian Sirtuins: Biological Insights and Disease Relevance

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Abstract

Aging is accompanied by a decline in the healthy function of multiple organ systems, leading to increased incidence and mortality from diseases such as type II diabetes mellitus, neurodegenerative diseases, cancer, and cardiovascular disease. Historically, researchers have focused on investigating individual pathways in isolated organs as a strategy to identify the root cause of a disease, with hopes of designing better drugs. Studies of aging in yeast led to the discovery of a family of conserved enzymes known as the sirtuins, which affect multiple pathways that increase the life span and the overall health of organisms. Since the discovery of the first known mammalian sirtuin, SIRT1, 10 years ago, there have been major advances in our understanding of the enzymology of sirtuins, their regulation, and their ability to broadly improve mammalian physiology and health span. This review summarizes and discusses the advances of the past decade and the challenges that will confront the field in the coming years.

Keywords

chromatin; metabolism; deacetylase; cancer; cardiovascular; inflammation

INTRODUCTION

It has been exactly 10 years since the *Silent Information Regulator 2* (*SIR2*) gene was shown to extend the life span of budding yeast by repressing genome instability (1,2). Since then, we have learned that *SIR2*-like genes, known as sirtuins, are found in most organisms, including plants, bacteria, and animals, and that they play key roles in promoting an organism's health and survival (3). Major strides have been made in understanding how sirtuins function at the molecular level to sense the amount of energy taken in, the timing of daylight, and stress from the environment and how they respond to such signals by inducing changes that promote survival during times of adversity. The discovery that sirtuins can carry out nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylation reactions (4) opened up a new line of investigation into the metabolic control of sirtuins and modulation of their activity by small molecules. Activation of sirtuins, either by restricting calories or by pharmacological means, extends the life span and promotes the health of a wide variety of organisms from yeast to mammals, thus demonstrating the feasibility of developing drugs that target the sirtuin and treat diseases of aging (5). This review covers the past 10 years of research into what sirtuins do at the molecular, cellular, and organismal levels to alter mammalian physiology. We also address the challenges still faced by researchers in the field.

SIRTUIN BIOLOGY

Identification of *SIR2/SIRT1* as a Regulator of Aging

The Sir2 protein, from the budding yeast *Saccharomyces cerevisiae*, is the founding member of a family of NAD^+-dependent deacetylases and ADP-ribosyltransferases, termed the sirtuins (6). The gene encoding the Sir2 enzyme, *SIR2*, was first identified by virtue of its role in the establishment of transcriptional silencing of mating-type loci in budding yeast. Subsequent studies have shown that *SIR2* is also pivotal for silencing at yeast telomeres and in the recombinant DNA (rDNA) (7,8), as well as in the partitioning of carbonylated proteins between mother and daughter cells (9).

The role of sirtuins in aging was initially discovered in yeast via a model of replicative life span, which measured the number of times a yeast mother cell produces a daughter cell before senescing. In 1997, a cause of yeast aging was identified as stemming from recombination at rDNA loci. The formation of an extrachromosomal rDNA circle (ERC) by homologous recombination is the starting event that leads to the amplification of ERCs in aging mother cells due to their replication and preferential segregation within mother cells (1). In 1999, addition of an extra copy of the *SIR2* gene was shown to extend replicative life span by ~30% by suppressing rDNA recombination and decreasing ERC formation while deleting *SIR2*-increased ERCs and shortened life span (2).

Since the late 1990s, it has become clear that the yeast *SIR2* gene is but one member of a large family of conserved genes found in organisms ranging from bacteria to mammals (10). The conserved functional role of *SIR2* in aging has become evident from studies involving more complex model organisms, such as *Caenorhabditis elegans* and *Drosophila*. In *C. elegans*, life span extension by *sir-2.1* requires the worm forkhead protein DAF-16 but may not require an intact insulin signaling pathway (11,12). Instead, *sir-2.1* binds to DAF-16 and activates it directly during stress (12). Moreover, *sir-2.1* does not appear to respond to changes in insulin signaling; rather, it is activated by stress treatments such as heat shock and oxidative damage. Increasing the copy number of the *SIR2* ortholog in *Drosophila* (*dSIR2*) also extends life span (13). Interestingly, overexpression of *dSIR2* specifically in neurons is sufficient to drive the increase in the fly's life span.

Mammalian Sirtuins: Enzymatic Activity and Regulation

The exciting discovery that increasing *SIR2* gene dosage in yeast, worms, and flies extends life span has spurred studies of mammalian sirtuins. Do sirtuins extend mammalian life span? Do mammalian sirtuins promote health and protect against aging-associated diseases? Are these proteins important for mediating benefits of calorie restriction (CR)? What are the targets of sirtuins? These are some of the central questions driving the field. Surprising progress has been made in only a decade of research on mammalian sirtuins. However, we are just beginning to understand the involvement of sirtuins in mammalian aging and age-associated diseases, and our comprehension of many sirtuins remains rudimentary.

Mammals contain seven sirtuins, SIRT1–7 (Table 1), which are categorized by their highly conserved central NAD^+-binding and catalytic domain, termed the sirtuin core domain (14). Although these sirtuins are relatively conserved, their N and C termini differ, and they are likely to have highly divergent biological functions owing to (*a*) different enzymatic activities, (*b*) unique binding partners and substrates, and (*c*) distinct subcellular localization and expression pattern (reviewed in Reference 15).

Reaction Mechanisms

The first reaction characterized for a sirtuin was a ribosyltransfer reaction catalyzed by the Sir2 homolog cobB from bacteria that convert 5,6-dimethylbenzimidazole to alpha-ribose-5,6-benzimidazole (16). This finding led to the surprising discovery that sirtuins are NAD^+-dependent deacetylases and ADP-ribosyltransferases. Most sirtuins catalyze NAD^+-dependent deacetylation (Figure 1) (4,17–19). SIRT4, however, possesses NAD^+-dependent mono-ADP-ribosyltransferase activity (20,21), whereas SIRT1 and SIRT6 have been shown to perform both auto-ADP-ribosyltransferase and substrate-specific deacetylase activities (22). Studies of SIRT4 and SIRT7 have not yet observed deacetylase activity, but such activity may require a specific substrate, as for SIRT6. Although yeast SIR2 is a histone deacetylase (HDAC), studies of sirtuins in other organisms have identified a host of nonhistone substrates. Indeed, sirtuins function by acting as substrate-specific protein deacetylases. Sirtuins perform deacetylation at modified lysine residues via a unique enzymatic mechanism that requires NAD^+ cleavage with each reaction cycle (4,18,23–25). Thus, unlike other HDACs that hydrolyze acetyl-lysine residues, sirtuin activity is intimately tied to the metabolic state of the cell.

The mechanistic basis for deacetylation by sirtuins is both elegant and novel. The deacetylation reaction begins with an amide cleavage of NAD^+ and the formation of nicotinamide (NAM) and a covalent ADP-ribose (ADPR) peptide-imidate intermediate (see Figure 1). The intermediate is resolved to form *O*-acetyl-ADP-ribose (AADPR), and the deacetylated substrate is released (25–27). Although the amide-to-AADPR acyltransfer is unfavorable, hydrolysis of NAD^+ can provide a favorable driving force for the overall sirtuin reaction (28–30). Although some details remain to be resolved, it is thought that peptide binding facilitates an allosteric change in enzyme structure that enables reaction of NAD^+ with a nucleophile from the enzyme to generate the enzyme-stabilized ADPR intermediate (30).

Sirtuin Modulation by NAD^+ Biosynthesis

There is increasing evidence that the biosynthetic pathways that generate NAD^+ are also critical for the regulation of sirtuins in various subcellular compartments. Both the Sternglanz and the Sinclair labs (31–33) reported that sirtuins are inhibited by NAM and in a noncompetitive manner with NAD^+. The surprising fact that it is noncompetitive inhibition led the authors to speculate that NAM binds to a novel site on sirtuins and is physiologically relevant, an idea that was subsequently verified. The mechanism of inhibition requires that NAM enter a highly conserved C-pocket adjacent to the NAD-binding site (31,34) and react with the peptide-imidate intermediate of the reaction, thereby regenerating NAD^+ (33). This type of reversible regulation is rare in biology and is thought to be a major mechanism of control for many of the sirtuins.

In yeast, worms, and flies, NAM is recycled back to NAD^+ in four steps, in the first of which it is catalyzed by Pnc1 to produce nicotinic acid. *PNC1* is upregulated in response to environmental stresses, such as heat and CR, leading to increased stress resistance and life span in *S. cerevisiae* and *D. melanogaster* (35–37). Thus, *PNC1* promotes survival and life span in response to environmental stress, which supports the view that life span extension by stress and diet is the result of an ancient survival response. Another NAD^+ precursor, nicotinamide riboside (NR), is found in yeast and mammalian cells and, when supplied exogenously to yeast, can also extend life span (38,39). Mammals recycle NAD^+ from NAM in two steps. First, a NAM phosphoribosyltransferase known as Nampt (also termed Visfatin or PBEF) converts NAM to nicotinamide mononucleotide (NMN) (40,41). Second, NMN is utilized by the isozymes Nmnat1, -2, and -3 to regenerate NAD^+ in the nucleus, Golgi, and mitochondria, respectively (42).

Consistent with the ability of *PNC1* to regulate Sir2 in yeast, mammalian Nampt is one of the main regulators of SIRT1 activity (40,43–45). Interestingly, the enzyme downstream of Nampt, Nmnat1, interacts directly with SIRT1 at promoters, indicating either that this enzyme hands off NAD^+ to SIRT1 or that there are nanopools of NAD^+ that influence SIRT1 activity (45).

The Nampt enzyme and NMN can also be found in the serum of mice and humans (where the enzyme is known as eNAMPT) (46). Imai and colleagues (47) proposed that NMN is a signaling molecule that allows stressed or nutrient-deprived cells to communicate with other parts of the body. This concept, termed the NAD world, is an area of considerable interest, especially given the possibility of using NMN or a downstream molecule such as NR as a therapeutic for type II diabetes mellitus (TDM) or other diseases of aging (47–49).

SIRT1 and Nampt form an essential part of the mammalian circadian clock feedback cycle. Nampt is under the transcriptional regulation of a CLOCK-BMAL-SIRT1 complex, which increases the conversion of NAM to NAD^+. This in turn activates SIRT1, which reactivates Nampt expression—all in a 12-h cycle (50–53). NAD^+ levels are dynamically regulated; thus, caution should be exercised when obtaining and comparing results from different times of the day.

In mammals, not only is NAD^+ destroyed by the sirtuins (and by poly-ADP-ribose polymerase), it is continually catabolized by CD38, a glycohydrolase. CD38 was first described as a NAD cyclase on the cell surface involved in immunity, but this is a relatively minor activity. Knockdown or deletion of CD38 increases steady-state levels of NAD^+, leading to speculation that inhibition of this enzyme could be an effective way to activate sirtuins (54,55). As we describe below, one-year-old mice lacking CD38 were protected from age-onset obesity and diabetes, perhaps because of increased sirtuin activity (54,55).

The AADPR produced by the sirtuin reaction is a novel metabolite that is less studied but that may have major physiological significance. For example, in yeast, AADPR promotes the assembly of the Sir complex, specifically Sir3 with the Sir2/Sir4 dimer, and catalyzes the spread of the SIR complex across chromatin (56). In mammals, AADPR binds and activates the transient receptor potential melastatin-related channel 2 (57). AADPR can also be metabolized by nudix hydrolases in vitro and may be metabolized in vivo to acetate by an unidentified enzyme (58). Thus, the cleavage products derived from NAD^+ during sirtuin-mediated deacetylation yield metabolites that may mediate biologically relevant functions in aging and metabolism. Given how little is known at this stage, this area of sirtuin biology may yield some of the more interesting discoveries in coming years.

Mammalian Sirtuins: Subcellular Localization

Mammalian sirtuins are found in numerous compartments within the cell (Table 1). SIRT1, -6, and -7 are found predominantly in the nucleus (59); SIRT3–5 reside in mitochondria; and SIRT2 is primarily cytoplasmic. The subcellular localization of these proteins probably depends upon cell type, stress status, and molecular interactions. For instance, SIRT1 and -2 were found to localize in both the nucleus and the cytoplasm and to interact with both nuclear and cytosolic proteins (60–62).

What governs sirtuin localization? Why is SIRT1 sometimes nuclear and sometimes cytosolic? First, each sirtuin contains primary amino acid signal sequences that contribute to its intracellular localization. For example, the nuclear localization of SIRT1, -6, and -7 is largely attributed to their nuclear localization signals. In addition to possessing two nuclear localization signal regions, SIRT1 contains two nuclear export signals (61). Thus, the exposure of nuclear localization signals versus nuclear export signals may dictate the cytosolic versus nuclear localization of SIRT1.

SIRT3–5 contain N-terminal mitochondrial targeting sequences and are widely believed to localize to the matrix of mitochondria (21,22,63–65). However, studies have not conclusively ruled out localization for these proteins to other compartments of mitochondria. The localization of SIRT3 has been hotly debated (66). Initial reports found that SIRT3 was localized exclusively to mitochondria (22,64). However, subsequent studies suggested that SIRT3 may translocate from the mitochondria to the nucleus during cellular stress (67,68). Insights into the function of SIRT3 may come from a better understanding of its structure and enzymology. A recent paper identified a SIRT3 crystal structure of the apoenzyme and a reaction-intermediate structure trapped by a thioacetyl peptide (69). This study demonstrated that the substrate induces conformational changes and that the acetylated peptide is the first substrate to bind to SIRT3, preceding NAD^+ (69). In sum, sirtuins are distributed among multiple compartments of the cell, and their localization may be dynamic, depending on tissue/ cell type and physiologic condition.

Sirtuins in Calorie Restriction

Numerous studies have shown that a diet of reduced calories, also known as CR, promotes life span extension by up to 50% in a wide range of organisms, including yeast, worms, flies, and mice (70). However, there are some exceptions, including wild mice and houseflies (71,72). In mammals, CR triggers physiological changes that improve glucose homeostasis: Rodents and humans on CR demonstrate decreased insulin and glucose levels and have improved insulin sensitivity. These metabolic changes are relevant to aging because decreasing insulin signaling is implicated in longevity regulation in studies of model organisms (73). The physiology of CR has been comprehensively reviewed elsewhere (70).

The discovery that Sir2 is a conserved regulator of life span in yeast, worms, and flies, coupled with the fact that sirtuin activity relies upon NAD^+, has intensified interest in elucidating the role of Sir2 in CR. Does Sir2 activity mediate CR benefits? The answers depend on the CR regimen (moderate versus severe restriction) and genetic background. In multiple studies of yeast replicative aging (the number of times a mother cell divides), life span extension by CR does require *SIR2*. Moreover, a moderate CR diet (0.5% glucose) was shown to increase mitochondrial respiration, upregulate Sir2 activity, and suppress rDNA recombination (36, 74). The yeast homologs of Sir2, Hst1 and Hst2, have also been shown to promote replicative life span (75).

However, there is some debate about the exact role of sirtuins in yeast CR. A more severe CR regimen (0.05% glucose) also extends yeast replicative life span but does not require *SIR2* or mitochondrial respiration (76,77). Lamming and colleagues (75) showed that deleting both *SIR2* and *HST2* blocked CR-mediated life span extension, suggesting that there is functional redundancy within the yeast sirtuin family. *SIR2* had no effect on yeast survival under starvation conditions (chronological life span) and appears rather to reduce survival of certain exceptionally long-lived mutant strains such as *sch9* (78). In worm models of CR, *sir-2.1* does not seem to be required for life span extension, although, given the variety of ways to perform CR in worms, *sir-2.1* could be required for a diet that has not yet been tested; alternatively, the other three worm sirtuins could be involved (79).

Because the amount of yeast Sir2 protein does not increase during CR (36), researchers have sought other explanations for increased Sir2 activity. The Guarente laboratory (80) proposed that during CR, a reduction in NADH (an inhibitor of SIR2) results in increased Sir2 activity, whereas the Sinclair and Smith labs (36,37,81,82) proposed that the increase in Sir2 activity is due to upregulation of *PNC1* during CR, which depletes NAM and increases flux through the NAD^+ salvage pathway. Interestingly, *PNC1* is also upregulated by mild stresses that extend life span such as increased temperature (37°C) and nitrogen restriction. These data are seen as evidence that CR is a form of hormesis, or a mild stress that induces a beneficial defense

response (83–85). The NADH and the *PNC1* mechanisms are fundamentally different: The former regulates sirtuins directly, whereas the latter also involves an active genetic pathway that responds specifically to stress. Since 2003, there has been increasing evidence for both these mechanisms of Sir2/SIRT1 regulation in yeast and in mammals, suggesting that they probably act in concert (32,86,87), although some investigators have questioned whether NADH is potent enough to inhibit SIRT1 in vivo (26).

These findings have generated considerable interest in elucidating the role of mammalian sirtuins in CR. So far, most of these studies have involved SIRT1 and provide support for the model that SIRT1 mediates several salutary effects of CR. First, rodent studies show that CR upregulates SIRT1 expression in a variety of tissues, such as brain, kidney, liver, white adipose, and skeletal muscle (88). Nonetheless, SIRT1 induction is not observed in all CR studies, and it may be induced in a tissue-specific manner or even decreased (89). Notably, NAD$^+$ levels are also increased in some tissues during CR. Thus, it is likely that in many tissues a combination of boosting SIRT1 protein levels and NAD$^+$ concentration (or flux though the NAD salvage pathway) contributes to increased activity of this sirtuin during CR.

Mouse models have also tightened the link between SIRT1 and CR. The Guarente lab (90) was the first to show that SIRT1 is required for the induction of a phenotype by CR: an increase in physical activity. Transgenic mice that over-express SIRT1 also display several metabolic benefits that overlap with CR phenotypes. For example, the first study using a knockin mouse model with SIRT1 expression driven by the beta actin promoter demonstrated that SIRT1-overexpressing mice are leaner and more glucose tolerant and that they display reduced levels of blood cholesterol, adipokines, and insulin compared to wild-type controls (91). In another series of elegant studies, wild-type or mutant SIRT1 was overexpressed in mice using its own promoter from a bacterial artificial chromosome. These animals were used to demonstrate that elevated SIRT1 did not improve basal glucose tolerance but rather attenuated obesity-induced glucose intolerance (92). Another study showed that transgenic SIRT1 mice are resistant to liver steatosis (fatty liver) and insulin resistance (93). These SIRT1 phenotypes are similar to the effects of treating mice with SIRT1 activators such as resveratrol and SRT1720 (94–96). Because many of these studies were performed with whole-body transgenic animals, it will be useful to identify the tissues that drive these protective phenotypes. Moreover, it will be interesting to know whether overexpression of SIRT1 can extend mean or maximum life span or merely health span.

SIRT1 null mice have also provided evidence that SIRT1 mediates aspects of CR. As mentioned above, the first study (90) investigated the behavior of mice fed a CR diet. CR causes an increase in activity in wild-type mice, which may aid animal survival in the wild by triggering foraging when food is scarce. Whole-body SIRT1 null mice do not show this increase, suggesting that SIRT1 is required for this phenotype of CR (90,97). Recently, life spans of SIRT1 null mice have been measured. SIRT1 null mice have a shorter life span than do their wild-type littermates, and CR does not increase the life span of these animals (98). These data provide important evidence that SIRT1 may be required for life span extension by CR in mammals. However, firm conclusions are complicated by the fact that SIRT1 null mice that survive to adulthood die prematurely and have developmental defects (99). Future life span studies that use SIRT1 tissue-specific knockout mice may ascertain which tissues drive this important phenotype. Notably, few studies have probed the role of SIRT2–7 in CR phenotypes, but these sirtuins may also play roles in regulating the physiologic responses to CR.

Posttranslational Regulation: Small Molecules, Interactions, and Modifications

Given that SIRT1 levels are responsive to environmental stimuli such as daylight, cell stress, and CR, it is not surprising that the gene is controlled by numerous transcription factors and

microRNAs, including p53, FOXO3, HIC1:CtBP, E2F1, c-Myc, miR-34, and miR-199 (100–109). Almost nothing else is known about how the other sirtuins are regulated at the transcriptional level.

As mentioned above, sirtuins are also regulated at the posttranslational level. The discovery that sirtuins can also be modulated directly by small molecules and by protein interactors has opened up the possibility of mimicking the benefits of CR without having to restrict calories. Small-molecule modulators of SIRT1 include the inhibitors splitomycin, sirtinol (110), and EX-527 (110), as well as analogs of sirtinol that inhibit yeast SIR2, SIRT1, and SIRT2 (Figure 2) (111). SIRT1-activating compounds (STACs) include resveratrol, SRT1720 and SRT2183, and analogs of NAM (94,112,113). With the exception of NAM analogs such as methyl-NAM, which interfere with NAM inhibition of sirtuins (32), SIRT1 activators work by lowering the Michaelis constant (K_m) for the substrate and for NAD$^+$ and, to a lesser extent, by increasing enzyme velocity (V_{max}) (94,113).

During the past decade, over 3500 STACs have been synthesized and analyzed (114).With regard to resveratrol and other natural polyphenols of similar structure, such as fisetin and butein (see Figure 2), these molecules have been shown to extend life span in a wide variety of organisms from yeast to flies to obese mice (115–118). However, some labs report little or no life span extension (119,120). The question remains: How do these molecules activate Sir2 and SIRT1? Assays used to detect activation by fluorescence or mass spectrometry have typically utilized peptide substrates that contain additional groups such as AMC (7-amino-4-methylcoumarin) or TAMRA (tetramethyl-rhodamine) (94,113,121), although these groups are not necessary to detect activation of SIRT1 by small molecules (122). Some studies have questioned whether this assay recapitulates SIRT1 activation on a native substrate, yet the increasing number of studies showing dependency of these molecules on SIRT1, and their ability to mimic CR and SIRT1 overexpression in vivo (95,112,116,123,124), argues that they act in large part via SIRT1 (92,93,96,125). Sauve (28) speculated that the fluorophore in the assay reproduces biophysical properties of native substrates in cells, thereby providing a cognate-binding site for compounds such as resveratrol in vivo.

A number of protein modulators of SIRT1 have also been identified, including the activators AROS (active regulator of SIRT1) (126), necdin (127), and DBC1 (the inhibitor deleted in breast cancer 1) (Figure 3) (128,129). Both DBC1 and AROS bind to the N terminus of SIRT1, the same region to which SIRT1 small-molecule activators bind, raising the possibility of a common mechanism of control. It is also clear that sirtuins can be modified directly. For example, SIRT1 has at least 13 residues that are phosphorylated in vivo, and removal of the phosphates decreases catalytic activity. Mutating two cyclin B/CDK1 sites (Thr530 and Ser540) disrupts the normal cell cycle and proliferation (130). These modulators of SIRT1 activity are described in more detail below in relation to their effects in specific disease models.

METABOLIC DISEASES

A growing area of sirtuin research involves the regulation of metabolism. Maintenance of energy homeostasis requires a coordinated regulation of energy intake, storage, and expenditure. In healthy individuals, fluctuations in any of these processes are compensated by adaptation by the other two. This elegant balance is orchestrated by the involvement of interorgan communication using endocrine and metabolic pathways. Thus, metabolic pathways are designed to sense incoming nutritional and environmental cues and to respond appropriately. Metabolic imbalance occurs when the ability to adapt to changes in energy intake, storage, or energy expenditure is lost. Abnormal metabolic homeostasis can have severe consequences and often manifests as syndromes such as obesity and TDM. Moreover, signs of metabolic imbalance, such as insulin resistance, are observed with increasing frequency

during aging. The identification of central pathways that regulate metabolic homeostasis is an area of intense sirtuin research and has important implications for understanding the molecular networks that control adaptation responses and for treating metabolic diseases. This section reviews our understanding of SIRT1 and mitochondrial sirtuins in regulating adaptation to nutrients.

There has been intense scrutiny of the role of sirtuins in metabolic pathways. Again, most of the studies have focused on SIRT1. Although the scope and detail of SIRT1 functions are not yet fully elucidated, overwhelming evidence suggests that this enzyme senses nutritional availability and relays this information to proteins that govern fuel utilization and energy adaptation. Multiple SIRT1 targets and regulatory mechanisms are highlighted in Figure 3. SIRT1 binds to and deacetylates a number of important transcription factors—such as peroxisome proliferator-activated receptor gamma (PPARγ), PPARα, PPAR gamma coactivator 1 alpha (PGC-1α), and the forkhead box, subgroup O (FOXO) family of transcription factors—to drive metabolic responses such as insulin secretion, gluconeogenesis, and fatty acid oxidation. SIRT1 thus acts as a pleiotropic energy sensor to help mount an appropriate physiological response.

Mitochondrial Biogenesis and Gluconeogenesis

In vivo studies that use mouse models support the implications of biochemical and cellular data. For example, PGC-1α seems to be a predominant SIRT1 target in a number of cell types. PGC-1α was originally identified and cloned as a cold-inducible PPARγ activator in brown adipose tissue (131). Now it is clear that PGC-1α is a potent inducer of mitochondrial biogenesis as well as of the uptake and utilization of substrates for energy production in a number of cell types. A series of elegant studies (132) first described the importance of the SIRT1–PGC-1α network in hepatocytes. SIRT1 can interact with and deacetylate PGC-1α in vitro (132). The deacetylation of PGC-1α results in the upregulation of its activity.

Activated SIRT1 deacetylates PGC-1α, resulting in the SIRT1-dependent induction of gluconeogenic genes and the suppression of glycolytic genes (132). The relevance for SIRT1 in gluconeogenesis in vivo was examined through use of adenovirus to decrease the levels of SIRT1, specifically in adult mouse liver (132). In this model, knockdown of SIRT1 in liver caused mild hypoglycemia and increased glucose production. Loss of hepatic SIRT1 in these adult animals actually improved glucose clearance and increased insulin sensitivity. Similarly, knocking down SIRT1 in adult rat liver decreased hepatic glucose production in a rat model of TDM (133). In these experiments, fasting was found to increase NAD$^+$ concentrations and SIRT1 levels in mouse liver (132). Several groups have also reported increases in hepatic NAD$^+$ during nutrient deprivation, such as in fasting and in CR in whole-cell extracts and in mitochondria (44).

Two other studies have utilized liver-specific SIRT1 null mice and observed no change in insulin or glucose homeostasis or in gluconeogenesis (89,134). What can account for the differences in these models? It could be that the absolute loss of SIRT1 in the null models activates compensatory pathways that restore gluconeogenesis. Although these mice appeared developmentally normal, perhaps the absence of SIRT1 during liver development activated compensation mechanisms. Another explanation is that studies of SIRT1 in the liver failed to induce the normal feedback loop provided by TORC2, which was recently shown by Montminy and colleagues to repress SIRT1-stimulated gluconeogenesis (135). Together, these data illustrate the complexity and difficulty of interpreting studies of isolated tissues in the mammalian system.

Substrate Usage: Glycolysis Versus Fatty Acid Oxidation

Later studies have shown that SIRT1 can orchestrate a coordinated shift in mitochondrial substrate usage, primarily by regulating PGC-1α activity. This switch allows the cell to preserve glucose while oxidizing fatty acids, reminiscent of the substrate utilization observed during nutrient deprivation. In skeletal muscle, SIRT1 deacetylates PGC-1α to promote transcription of mitochondrial fatty acid oxidation genes, and SIRT1 is required for the switch to fatty acid oxidation under low-nutrient conditions (136–139). Additionally, activation of PGC-1α triggers the transcription of oxidative phosphorylation genes, suggesting that SIRT1 may couple fatty acid oxidation with energy production (140). Consistent with these findings, liver mitochondria isolated from whole-body SIRT1 null mice displayed lower rates of respiration and reactive oxygen species (ROS) production (97). Moreover, SIRT1 null mice had increased levels of active AMP-activated protein kinase (AMPK) after a 24-h fast, suggesting a decrease in their ATP levels (97). Thus, activation of PGC-1α may provide a direct mechanism through which SIRT1 can control mitochondrial biogenesis.

As in skeletal muscle, SIRT1 activation causes a global shift toward hepatic fatty acid oxidation during nutrient deprivation (134,138). SIRT1 may act through PGC-1α to activate PPARα transcription to induce fatty acid catabolic genes (134). SIRT1-overexpressing transgenic mice gain weight, similar to wild-type controls, but display protection from hepatic steatosis when fed a high-fat diet (93), consistent with the effects observed for the SIRT1 activators resveratrol and SRT1720 (96,138,141,142). SIRT1 null hepatocytes exhibit lower rates of fatty acid oxidation (134). Moreover, when liver-specific SIRT1 knockout mice were fed a high-fat, Western-style diet, they gained more weight than did littermate controls and exhibited higher lipids and decreased ketones after fasting (134).

Again, some evidence is conflicting. One study (143) demonstrated that similarly generated liver-specific SIRT1 null animals gained less weight when fed a high-fat diet. Another study (89) suggested that, because SIRT1 activates liver X receptor (LXR), lack of LXR activation in the SIRT1 liver-specific null animals decreased fat production in these mice, providing one mechanism for absence of weight gain on a high-fat diet. Finally, these findings suggested that SIRT1 activity is (*a*) increased in liver by a high-fat diet and (*b*) decreased in this organ by CR. Thus, it is entirely possible that sirtuin activity (or NAD$^+$) increases in some tissues during fasting but is actually repressed in others. How can these two studies be reconciled? Both used C57BL/6 mice, so the best explanation is that differences in dietary composition may account for some discrepancies. Indeed, the latter study used considerably more carbohydrate than the other did.

Importantly, other studies have linked SIRT1 with improved glucose and insulin homeostasis and fuel utilization. For example, pharmacological activation of SIRT1 in vivo by resveratrol or by more potent SIRT1 activators (e.g., SRT1720 and SRT2183) induces changes in gene expression consistent with those induced by PGC-1α activation in liver and muscle, promotes mitochondrial respiration, and increases insulin sensitivity (94–96). Here, the lack of gluconeogenesis is probably the effect of TORC2 repressing PGC-1α in the liver (135), an effect that could be absent in liver-specific genetic alterations. Consistent with this finding, blocking SIRT1 leads to insulin resistance in cultured cells (124). The authors suggest that SIRT1 represses protein tyrosine phosphatase 1B, a negative regulator of insulin signaling, providing a potential mechanism for the effect of SIRT1 on insulin signaling in peripheral tissues (124).With regard to the starvation response, it is also notable that SIRT1 is required for full induction of autophagy (144), but follow-up in vivo studies are needed to assess the relevance of this finding to metabolism or cell survival.

AMPK-SIRT1 Axis

Exercise induces an energy deprivation state that activates similar metabolic pathways in skeletal muscle. Upon exercise, AMPK was found to boost SIRT1 activity by increasing intracellular NAD^+ (87). In turn, SIRT1 deacetylates and activates transcription programs driven by PGC-1α, FOXO1, and FOXO3. These findings help to explain many of the overlapping functions of AMPK and SIRT1 activation (Figure 4). Pharmacological activation of SIRT1 by SRT1720 does not activate AMPK directly, but it does increase insulin sensitivity, improve endurance, increase respiratory quotient, and shift muscle toward oxidative pathways (138). Thus, the benefits of SIRT1 activation can occur in the absence of AMPK activation, but clearly the two pathways can act in concert to reinforce one another.

Insulin Secretion

In addition to modulating insulin and glucose homeostasis in liver and muscle, SIRT1 acts in pancreatic beta cells directly. SIRT1 is a positive regulator of insulin secretion, which triggers glucose uptake and utilization. Through use of a mouse model in which SIRT1 was overexpressed specifically in pancreatic beta cells (BESTO mice), it was shown that elevated levels of SIRT1 increased glucose-stimulated insulin secretion ex vivo (145). These mice consistently demonstrated improved glucose tolerance in vivo. In line with studies of BESTO mice, whole-body SIRT1 null animals demonstrated impaired insulin secretion in ex vivo studies using isolated islets (146). In both models, it was shown that SIRT1 promotes insulin secretion by suppressing uncoupling protein 2 (UCP2) expression, which could lead to a boost in cytosolic ATP/ADP ratios. Although both studies demonstrated that levels of SIRT1 protein regulate insulin secretion, elevated levels of SIRT1 protein alone were not sufficient to promote insulin secretion, as was illustrated by studies of aging BESTO mice. The increase in insulin secretion in BESTO mice declined with age but could be restored by addition of NMN. Furthermore, a subsequent study showed that a decline in NAD^+ biosynthesis may be responsible for the decreased SIRT1 activity in these islets during aging (147). This finding raises the importance of investigating NAD^+ biosynthesis, not sirtuin levels alone, during aging in other metabolic tissues.

Fat Tissue

SIRT1 also regulates white adipose tissue (WAT), which is significant because fat accumulation and distribution increase during aging. WAT functions both to store fats and to serve as an endocrine organ by secreting hormones, such as (*a*) leptin, (*b*) adiponectin, and (*c*) inflammatory agents such as tumor necrosis factor alpha (TNFα) and resistin. An increase in adiposity also elevates the risk of developing metabolic syndromes and insulin resistance, and a reduction in WAT mass is presumed to be responsible for at least part of the life span increase by CR. In cultured cells, SIRT1 has been shown to bind PPARγ and repress transcription of its target genes involved in fat storage (148). As a result, upregulation of SIRT1 in differentiated fat cells triggers lipolysis and results in decreased fat storage. Consistent with these findings, treatment of mice on a high-fat diet with resveratrol or SRT1720 reduces weight gain (94,96). Thus, an increase in SIRT1 levels may be a part of the metabolic program to mobilize fatty acids during fasting and to reduce adiposity in WAT during CR. SIRT1 also contributes to the production of adiponectin by WAT by enhancing the interaction between FOXO1 and C/EBP(149). Because adiponectin improves insulin sensitivity, its control by SIRT1 may provide yet another mechanism for regulating metabolic homeostasis.

Mitochondrial Sirtuins in Metabolism

SIRT3–5 are the sirtuins that reside in the mitochondria—a metabolic hot spot (21,35,42,55). Mitochondria are at center stage for cellular energy (ATP) production, generation of ROS, and signaling during apoptosis. These organelles consume 85%–95% of the oxygen used by cells

in a series of enzymatic reactions that ultimately generate ATP from oxidative phosphorylation. When energy generation is required, mitochondrial biogenesis is often accompanied by an increase in oxidative fuel utilization to promote energy production. Mitochondria serve as a nexus for nutrient adaptation (Figure 5). Pyruvate, derived from glucose, is metabolized via the tricarboxylic acid (TCA) cycle; fatty acids and amino acids are also converted in the mitochondria by fatty acid oxidation and amino-transferase reactions, respectively. Not surprisingly, defects in mitochondrial functions have been linked to aging and can result in imbalances in metabolic homeostasis (150). For instance, impaired mitochondrial function in the pancreas could inhibit the increase in ATP/ADP needed to stimulate insulin exocytosis, and defective mitochondria in muscle may lead to insulin resistance (151).

Recent studies have revealed that mitochondrial sirtuins could be a pivotal part of the mitochondrial changes driving energy adaptation. However, compared with SIRT1, little is known about the biochemical and biological functions of SIRT3–5. SIRT3 is the mitochondrial sirtuin most conserved with SIRT1, and it is the best-understood mitochondrial sirtuin. SIRT3 is expressed in all tissues, with the highest levels appearing in metabolically active tissues such as brown adipose, muscle, liver, kidney, heart, and brain (64,152,153).

Experiments comparing membrane-bound and -soluble mitochondrial fractions have suggested that SIRT3 is in the matrix and in the inner mitochondrial membrane. Moreover, electron microscopy studies have observed SIRT3 together with mitochondrial cristae, the site for oxidative phosphorylation. SIRT3 null mice do not have an overt developmental or metabolic phenotype (154). The mice display normal body composition and levels of mitochondrial proteins and a typical response to fasting and cold exposure, but they also display a robust biochemical phenotype—increased levels of mitochondrial acetylation (154). This observation is significant because metabolic proteins, such as TCA-cycle enzymes, fatty acid oxidation enzymes, and subunits of oxidative phosphorylation complexes, were found to be heavily acetylated in a mass spectrometry survey of acetylation. However, it remains to be determined precisely how global acetylation influences mitochondrial control of metabolism. Notably, no mitochondrial acetyltransferase has yet been identified.

SIRT3 can bind and deacetylate at least three metabolic substrates in the mitochondria: acetyl-CoA-synthetase (AceCS) (65,155), glutamate dehydrogenase (GDH), and complex I. SIRT3-mediated deacetylation seems to have an activating effect on enzymatic activity. For example, two groups (154,156) have shown that SIRT3 deacetylates and activates AceCS, which forms acetyl-CoA from acetate, CoA, and ATP. Mammalian cells do not require AceCS to synthesize acetyl-CoA under normal conditions. However, AceCS activity becomes critical under ketogenic conditions, such as prolonged fasting and diabetes, when energy-expending tissues such as muscle must convert acetate released by the liver into acetyl-CoA for further metabolism. SIRT3 also binds and deacetylates GDH, although an effect on its activity has not been reported (154,156). GDH interconverts glutamate to alpha-ketoglutarate, and its regulation by SIRT3 could be a way to control amino acid flux into the TCA cycle. GDH activity also increases during fasting and CR in liver, but the role of SIRT3 in this response has not been investigated. The regulation of AceCS and GDH in vitro by SIRT3 warrants further study to evaluate whether SIRT3 regulates acetate or amino acid metabolism in vivo. These interactions suggest that under conditions of energy limitation SIRT3 may play a role in funneling carbons from alternative sources—namely ketone bodies and amino acids—into the central metabolism of the TCA cycle. Consistent with this hypothesis, SIRT3 expression in brown adipose tissue and WAT increases during CR and decreases in genetically obese mice (153).

In addition to potentially regulating central pathways of mitochondrial metabolism, SIRT3 is linked to mitochondrial respiration. SIRT3 binds to complex I and promotes NADH-driven

mitochondrial respiration (157). Furthermore, mitochondria from SIRT3 null livers demonstrated decreased oxygen consumption, and heart, kidney, and liver displayed a 50% reduction in basal ATP levels (157). Overexpression of SIRT3 in brown adipocytes increases oxygen consumption, reflecting heightened electron transport activity and increased uncoupling (153). Accordingly, SIRT3 overexpression results in decreased membrane potential and ROS production in these cells (153). However, these overexpression studies are difficult to interpret because they were performed using a truncated form of SIRT3 that does not correctly localize to mitochondria (158).

Given the ties among SIRT3, metabolism, and mitochondrial function, it is especially intriguing that genetic studies have linked SIRT3 to human life span. A silent G–T transversion in the conserved sirtuin core domain is associated with survivorship in elderly males, and SIRT3 contains a variable number tandem repeat polymorphism found almost exclusively in males over the age of 90 (159,160). Future biochemical studies to examine how SIRT3 single nucleotide polymorphisms modulate protein level or activity will be critical for a better understanding of these associations. Although these human genetic studies are limited in scale, the findings hint that SIRT3 may have a positive impact on human life span.

SIRT4 localizes to mitochondria of human and mouse cells and has been observed in the mitochondrial matrix (21,22,161). *SIRT4* is a ubiquitously expressed gene, but in mice its protein levels are highest in the kidney, heart, brain, liver, and pancreatic beta cells (21,22, 161). Unlike SIRT3, SIRT4 does not have a detectable NAD^+-dependent deacetylase activity toward canonical sirtuin targets (21,22,161). It is possible that SIRT4 is more specific in its substrate specificity than SIRT3, and future surveys may identify specific substrates that are deacetylated by SIRT4. However, SIRT4 has been shown to contain a NAD^+-dependent ADP-ribosyltransferase activity. One SIRT4 substrate has been identified; SIRT4 interacts with and represses GDH activity via ADP-ribosylation (21). GDH regulates the usage of amino acids into energy production. Its biological significance is evident from patients with GDH-activating mutations who exhibit hyperinsulinism/hyperammonia syndrome (162). Isolated pancreatic islets from SIRT4 null mice exhibited higher GDH activity and increased insulin secretion in response to glucose and amino acids. In a separate study, SIRT4 overexpression in insulinoma cells suppressed insulin secretion (161). SIRT4 has also been shown to interact with insulin-degrading enzyme and adenine nucleotide translocator, but the functional significance of these interactions is not known (161). It will be important for future studies to address whether SIRT4 affects fuel utilization in other tissues. Also, how SIRT4 function relays physiological changes during nutrient limitation or metabolic dysfunction remains to be evaluated.

Recently, the first comprehensive study of SIRT5 revealed that this sirtuin regulates ammonia entry into the urea cycle. SIRT5 localizes to the mitochondrial matrix and is ubiquitously expressed. SIRT5 functions as a weak NAD^+-dependent deacetylase. SIRT5 null mice have been generated and are developmentally normal without obvious metabolic defects (63,154). SIRT5 is known to interact with at least two proteins involved in cellular metabolism: cytochrome *c* and carbamoyl phosphate synthetase 1 (CPS1) (63,156). CPS1 is the rate-limiting first step of the urea cycle; its activity is required for clearing ammonia generated by amino acid metabolism. By deacetylating CPS1, SIRT5 stimulates its enzymatic activity (63). Mice lacking SIRT5 displayed elevated ammonia levels after a prolonged fast, suggesting that this sirtuin helps the liver deal with by-products of amino acid metabolism (63). It remains to be seen whether loss of SIRT5 also increases susceptibility to ammonia toxicity.

The regulation of mitochondrial sirtuins is an area of increasing interest. There are three mechanisms of regulation: First, mitochondrial NAD^+ levels can rise due to increases in Nampt by up to twofold in the liver of fasted rats, with a concomitant increase in stress resistance

(44,163). Second, mitochondrial sirtuins are also regulated at the protein level: SIRT3 protein levels increase during CR, fasting, stress, and exercise. (153,163,164). Third, mitochondrial sirtuins are regulated by localization. For example, SIRT3 may translocate from the nucleus to the mitochondria upon cellular stress (67,68), and SIRT5 is translocated predominantly into the mitochondrial intermembrane space or the matrix, depending on the stimulus (156).

An elegant coordination of metabolism by mitochondrial sirtuins is emerging. SIRT3–5 appear to work in concert to regulate critical aspects of mitochondrial metabolism during times of adaptation. For example, both SIRT3 and -4 regulate GDH and may stimulate ammonia production. Accordingly, SIRT5 helps to clear this ammonia by activating the urea cycle. Future studies will need to fill in the many gaps in our current understanding of how mitochondrial sirtuins regulate metabolism. SIRT4 represses GDH, whereas the effect on SIRT3 is unknown. Are SIRT3 and -4 activated to function at the same time? In sum, these sirtuins appear to function at critical junctions in mitochondrial metabolism by acting as switches to facilitate fuel entry into the TCA cycle and by regulating oxidative phosphorylation.

CANCER

An area of considerable debate is the role of sirtuins in tumorigenesis and cancer cell proliferation. One of the confusing aspects of SIRT1 is that it plays a dual role in cell survival and cell death and can be modulated in different directions by a variety of different stimuli (Figure 6). Although CR is arguably the most effective way to prevent cancer in rodents and primates, which some view as an indication that sirtuins are tumor suppressors (70), some sirtuins, such as SIRT1 and -3, have prosurvival functions, which could be a sign that they promote tumorigenesis (165,166).

Initially, most of the evidence was in favor of SIRT1 being an oncogene, beginning with the identification of the first substrate of SIRT1: the tumor suppressor p53 (167,168). Deacetylation of lysine 382 on p53 by SIRT1 reduces p53 transactivation, allowing cells to bypass p53-mediated apoptosis. These early data, combined with other studies showing that SIRT1 can suppress apoptosis (88), implied that SIRT1 might be oncogenic. Recent work, however, shows that SIRT1 plays a critical role in DNA-break repair and can prevent tumorigenesis in mouse models of cancer. Mice with additional copies of SIRT1 do not show signs of premature death or increased tumorigenesis; in fact, in models of leukemia and colon cancer, SIRT1 transgenic mice live longer (169–172). These findings have led to considerable debate as to whether inhibition or activation of SIRT1 plays a role in the development of cancer (171).

How can we resolve the apparently contradictory data on SIRT1 in cancer? It may come down to the genetics of the tumor and the stage of tumorigenesis being assessed. In assessing the potential role of sirtuins in cancer and its treatment, however, it is important to remember that most of the data so far have been generated through use of cell lines, which can be misleading when extrapolating to the in vivo situation. It is also important to recognize that some cancer models, such as the p53 lymphoma model used in SIRT1 studies, are more relevant to cancer prevention than to treatment. One reason for this finding is that SIRT1 is situated within feedback loops that allow it to promote cell survival in critical cell types without causing cancer. If and how this actually occurs are an area of major interest and will require considerably more investigation of SIRT1, not only in cell culture but also in vivo.

Altered Sirtuin Expression in Cancer

To date, the majority of information about the expression levels of sirtuins in cancer comes from studies of SIRT1 and, to a lesser extent, SIRT2. SIRT1 expression is relatively higher in many different types of cancer, including colon, skin, breast, and prostate cancers and leukemia,

compared to the corresponding normal tissue (121,173–177). But the correlation is not clear-cut. Wang et al. (178) analyzed a public database and found reduced levels of SIRT1 mRNA in prostate and bladder carcinoma, glioblastoma, and ovarian cancer compared to normal tissues. They observed significantly lower levels of SIRT1 mRNA in cancers with mutated BRCA than in cancers with wild-type BRCA1. Positive correlation between BRCA1 and SIRT1 expression was also observed through immunohistochemical staining of 45 human breast cancer samples.

A recent study of pancreatic cancer observed an increase of SIRT1 in only 1 out of 11 tumors (179). Indeed, there was a greater correlation between tumor tissue and the expression levels of the other sirtuins: SIRT2 (6 of 11), SIRT3 (4 of 11), and SIRT6 (4 of 11). The most striking result in this study was that SIRT5 transcripts were elevated in 10 of the 11 pancreatic cancers. All the patients in stages 0, IB, IIB, and IV had increased levels of SIRT5 mRNA, whereas one patient in stage III showed SIRT5 downregulation.

With regard to SIRT2, its role in regulating the cell cycle in response to stress suggests a possible role in tumorigenesis and perhaps some utility in chemotherapy. The latest evidence suggests that SIRT2 functions to release mitotic arrest in critically damaged cells, allowing them to proceed to apoptosis (17,180,181). Decreases in SIRT2 expression are common in gliomas and glioma-derived cell lines, and these decreases were traced to histone modifications at the promoter or deletion of the *SIRT2* gene. Thus, inhibition of SIRT2 may predispose cells to uncontrolled growth (182). In agreement with this hypothesis, a recent study found that lysine 56 of histone H3, a target of SIRT1/SIRT2, is hyperacetylated in cancer tissue. Furthermore, nontumorigenic MCF10A cells show much lower levels of acetylated H3K56 than do tumorigenic MCF7 breast cancer cells (183).

Together, these data indicate that decreased SIRT1/SIRT2 activity correlates with tumorigenesis and that increasing activity of SIRT2 in certain cancers could be beneficial. Cell biology experiments also support this view. Ectopically expressed wild-type SIRT2 slows cell-cycle progression and increases the number of multinucleated cells (60,180). Growth inhibition is dependent on the phosphorylation of serine 368 on SIRT2 by CDK1 (60,180). SIRT2 overexpression also arrests the cell cycle prior to entry into mitosis in response to microtubule inhibitors such as nocodazole (181,184). Conversely, SIRT2 inhibition promotes centrosome fragmentation and prolongs chronic mitotic arrest in response to nocodazole, but in so doing prevents cell death during reentry into the cell cycle (181). Interestingly, the SIRT2 catalytic mutant also increases the number of multinucleated cells (60,180), indicating that precise levels of SIRT2 are required for mitotic fidelity and that, depending on the circumstances, an inhibitor or an activator may be useful in treating certain cancers in vivo.

Increased levels of SIRT3 and -7 mRNA have been associated with node-positive breast cancer, compared with nonmalignant breast tissue (185). A reported increase in what was thought to be SIRT8 expression in thyroid cancer turned out to be an increase in SIRT7 expression (it should be noted that SIRT8 does not exist) (186). In summary, there is no definitive correlation between a sirtuin and any particular cancer, with perhaps the exception of a lack of SIRT1 in BRCA1-negative breast cancers, and the sample sizes used to investigate the possible association of the other sirtuins with particular cancers is still too small to draw meaningful conclusions.

SIRT1 as a Potential Oncogene

In cell culture experiments, a wealth of data show that SIRT1 can prevent apoptosis and senescence (62,128,187,188), suggesting that the inhibition of SIRT1 may be beneficial in treating some types of cancer in vivo (128,165,189). In 2001, SIRT1 was shown to interact with and target p53 for deacetylation, which reduces p53's DNA-binding capacity (102,168,

190). Cells with additional SIRT1 typically have lower levels of acetylated p53 lysine 382 in response to DNA damage and cell stress and are, in general, more resistant to p53-dependent cell-cycle arrest and apoptosis (167,168). Consistent with this finding, expression of the H363Y dominant-negative allele of SIRT1 or small interfering RNA (siRNA) to SIRT1 mRNA increases p53-dependent transcriptional activity (167) and increases the sensitivity of cells to stress (171,191). Similarly, cells derived from *SIRT1* knockout mice or cells treated with siRNAs against SIRT1 show high levels of hyperacetylated p53 on lysines 382 and 320 (188,192). The sirtuin inhibitor, sirtinol, induces senescence-like growth arrest and increases expression of plasminogen activator inhibitor 1 in human breast cancer MCF7 cells and lung cancer H1299 cells (193). Growth arrest is accompanied by impaired activation of c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase in response to epidermal growth factor and insulin-like growth factor I, whereas Ras is reduced. Tyrosine phosphorylation of the receptors for epidermal growth factor and insulin-like growth factor I and activation of protein kinase B are unaltered by sirtinol treatment. The same group (194), in studies of endothelial cell function, showed that SIRT1 inhibition increases p53 acetylation and induces premature senescence-like phenotype, whereas overexpression of SIRT1 prevents senescence-like changes. Others have reported that specific inhibition of SIRT1 by siRNA knockdown can increase tumor cell death with no toxic effect on normal cells in culture (188). In the latter study, however, the mechanism of cell death remains unclear because p53 is not essential for tumor cell killing by SIRT1 depletion, although it does not rule out participation by p53 (188).

Increased SIRT1 expression in human fibroblasts has been shown to promote cellular proliferation, reduce cellular senescence, increase the growth rate, and increase the cellular life span of 2Bs human embryonic lung fibroblasts, while reducing the expression of p16^{INK4A} and promoting phosphorylation of Rb (195). The authors (195) provided evidence that senescence was induced via the activation of ERK/S6K1, which occurred after overexpression of SIRT1 or treatment with resveratrol, a SIRT1 activator. Overexpression of SIRT1 has also been shown to repress the expression or activity of tumor-suppressor and DNA-repair genes, including *FOXO1/-2/-4, WRN, Rb, p73, MLH1*, and *NBS1* (reviewed in References 171 and 196). A recent study of zebrafish and mouse embryos demonstrated a role for SIRT1 in promoting angiogenesis (197,198), another indication that SIRT1 has effects that could be tumor promoting.

A study by Baylin and colleagues (172,199) indicates that SIRT1-mediated silencing of E-cadherin may play a role in tumorigenesis. The E-cadherin CpG island is frequently silenced by hypermethlylation in epithelial cancers. If a DNA break is initiated within the CpG island, SIRT1 appears to be required for the transient recruitment of DNA methyltransferase 3B and its subsequent silencing by methylation of the DNA. This result is consistent with recent studies linking SIRT1 to the repair of broken DNA (170,172,178,200), but whether SIRT1 can promote epithelial cancers via this mechanism in vivo remains to be determined. Testing the effects of SIRT1 inhibitors in such cancers may be of interest, particularly if the silencing by DNA methylation proves to be reversible.

Two tumor suppressors have been identified as negative regulators of SIRT1. A recent paper showed that hypermethylated in cancer 1 (HIC1), a zinc-finger/BTB domain protein regulated by p53, is a binding partner of SIRT1 that, in turn, represses the transcription of the *SIRT1* gene (102). Inactivation of HIC1 up-regulates SIRT1 transcription, thereby inactivating p53, allowing cells to bypass apoptosis after DNA damage. Importantly, the authors found that the HIC1 promoter undergoes hypermethylation during aging, which may lead to upregulation of SIRT1 during aging and, therefore, susceptibility to cancer. Notably, inhibition of glycolysis decreases the binding of the corepressor CtBP to HIC1, which increases SIRT1 expression, linking redox status to SIRT1 expression (103).

Another tumor suppressor that negatively regulates SIRT1 is DBC1 (128,129,201). The DBC1 protein forms a stable interaction with the N terminus of SIRT1 that inhibits SIRT1 deacetylase activity. Knockdown of DBC1 by siRNA promotes the deacetylation of p53 and allows cells to survive genotoxic stress, an effect that depends on SIRT1. These data indicate that DBC1 may promote breast cancer in part by activating SIRT1, thereby downregulating p53 and/or other tumor-suppressor pathways.

SIRT1 as a Potential Tumor Suppressor

Although cell-based studies have indicated that *SIRT1* may act as a tumor promoting gene, recent studies indicate that *SIRT1* can act as a tumor suppressor. First, SIRT1 knockdown, the catalytically inactive H363Y allele, or specific inhibition of SIRT1 in cells does not affect cell viability or cell growth and is not sufficient to induce activation of endogenous p53 (110, 188,195). In the Solomon et al. study (110), there was no increase in cell death despite DNA damage and increased p53 acetylation. Second, in cell culture studies, Mayo and colleagues (202) showed that SIRT1 stimulates TNFα-induced cell death, indicating that SIRT1 can promote apoptosis, not simply suppress it.

A recent study has highlighted a crucial feedback loop that could explain how SIRT1 could suppress oncogenesis in vivo (109). The c-Myc gene encodes a proto-oncogenic transcription factor that regulates cell proliferation, cell growth, apoptosis, and stem cell self-renewal. c-Myc binds to the SIRT1 promoter and induces SIRT1 expression, but SIRT1 then interacts with and deacetylates c-Myc, resulting in decreased c-Myc stability. This c-Myc-SIRT1 feedback loop could prevent cellular transformation and is consistent with a role for SIRT1 in tumor suppression.

In the first study to test whether SIRT1 promotes cell survival or death (i.e., oncogenic or tumor-suppressing activity) in an animal, SIRT1 was overexpressed in a mouse model of colon cancer, $APC^{min/+}$ (169). In this model, loss of the remaining wild-type copy of the *min* gene results in the relocalization of beta catenin to the nucleus, activating transcription of genes such as *myc* and *cyclin D1*, which in turn activate the cell cycle. CR has been shown to reduce tumor formation (203), as has resveratrol (204,205), but it was unclear whether SIRT1 overexpression would result in more or fewer tumors. Mice overexpressing SIRT1 in the small intestine and colon showed a four-fold reduction in size and number of adenomas. Ki67 and TUNEL staining showed that the tumors in the SIRT1-overexpressing mice were growing slower and undergoing more apoptosis. Deacetylation of beta catenin by SIRT1 was the favored hypothesis for the mechanism. Together, these data indicated that SIRT1 activation may have therapeutic potential in colon cancer and other tumors driven by beta catenin signaling.

Mice that are heterozygous for p53 have been extensively used to study genomic instability in mice (206). When exposed to ionizing irradiation, $p53^{+/-}$ mice show accelerated tumor development due to increased loss of heterozygosity at the p53 locus (207). Given that SIRT1 downregulates $p53$ in cell culture experiments, SIRT1 activity was expected to exacerbate the $p53^{+/-}$ phenotype and shorten life span, but the opposite occurred. In one study, resveratrol-treated animals showed a 24% increase in survival and a ~45% reduction in the frequency of fatal thymic lymphomas. In accordance with protection from irradiation-induced tumorigenesis, the overall tumor spectrum was highly reminiscent of nonirradiated $p53^{+/-}$ mice (206,208). Next, SIRT1 was overexpressed in thymocytes in a $p53^{+/-}$ model; again, after irradiation, the mean survival of the SIRT1-overexpressing mice was ~46% greater than in control animals, and the frequency of fatal thymic lymphomas was reduced by 45% in MISTO (MX-cre SIRT1-overexpressing) mice.

In a complementary study, SIRT1 null mice were crossed to the same $p53^{+/-}$ strain (109). On the basis of the cell culture data showing that SIRT1 downregulates $p53$, it was expected that

the $SIRT1^{-/-}$ $p53^{-/+}$ combination could delay the formation of cancer relative to $p53^{+/-}$ mice. In contrast, the $SIRT1^{-/-}$ mice experienced accelerated tumorigenesis, with multiple tumor types developing spontaneously from approximately 5 months of age. At 20 months of age, 76% of the $SIRT1^{-/-}$ $p53^{-/+}$ mice had tumors compared to approximately 10%–15% of the controls. Karyotyping of primary tumors showed aneuploidy and chromosomal aberrations (178). Resveratrol, a SIRT1 activator, delayed tumorigenesis and extended life span in this strain in two independent studies (170,178). Moreover, the relocalization of SIRT1 in response to DNA damage resulted in the absence of SIRT1 from promoters and changes in gene expression that occur during aging (170). These data support the RCM (relocalization of chromatin modifiers) hypothesis of aging, which states that the DNA damage–driven relocalization of chromatin-modifying proteins results in gene-expression changes that cause aging (170,209). However, further work is required to validate this model (Figure 7) (196).

$SIRT1^{-/-}$ mice experience embryonic lethality in inbred strain backgrounds (e.g., Reference 129). Based on the cell culture data showing that SIRT1 inactivates p53, it was initially suspected that p53 hyperactivity was responsible for the embryonic lethality of $SIRT1^{-/-}$ mice. Thus, deletion of p53 in the SIRT1 knockout mice was predicted to rescue the lethality of the knockout, but this was not the case (178,210). In their paper entitled "Sirt1 fails to affect p53-mediated biological functions," McBurney and colleagues (210) failed to find alterations in p53-downstream genes in an analysis of embryonic tissue from the SIRT1 knockouts.

In support of a tumor-suppressor function for SIRT1, the gene has been shown to play important roles in repairing broken DNA and maintaining genome stability. The first clue came in 1999, when SIR2 from *Saccharomyces cerevisiae*, the founding member of the sirtuin family, was shown by three independent laboratories to localize to broken DNA in response to DNA damage (211–213). This response required only a single DNA break and checkpoint signaling via RAD9 and MEC1 (the yeast ATR/ATM). At the time, it was unclear how SIR2, a deacetylase, was directly involved in DNA-break repair, if at all (214). The SIR2, -3, and -4 proteins were later shown to bypass G2 arrest with an unrepaired DNA break (215), and a study by Tyler and colleagues (216) showed that SIR2 binds to a double-strand-break repair site approximately 3 h after initiation of the break coincident with decreases in histone acetylation. These data were interpreted as evidence that SIR2 prepared chromatin around the break site to facilitate the repair process (216).

Four years later, a series of papers showed that mammalian SIRT1 regulates a critical repair factor in the MRE11/RAD50/NBS1 complex, NBS1 (217). Within months thereafter, it was reported that SIRT1 localizes to DNA breaks and is required for efficient DNA-break repair (170,178). Consistent with this model, cells and mice lacking SIRT1 are more prone to DNA damage–induced aneuploidy, and the efficiency of the repair of a DNA break is reduced by approximately 50% (170). Cells lacking SIRT1 are defective in recruiting DNA-repair factors to the break in response to ATM-and H2AX-mediated signals, including NBS1 and RAD51. These data may explain why mice overexpressing SIRT1 are less prone to loss of heterozygosity at the p53 locus and subsequent tumorigenesis and why $SIRT1^{-/-}$ mice are more prone (171). They may also explain, in part, why SIRT1 can suppress adenomas in $APC^{min+/-}$ mice that are caused by loss of heterozygosity at the *min* locus. However, this finding may not be the entire explanation for the reduced adenomas, because SIRT1 overexpression also increases apoptosis and reduces the number of mitoses in the tumors (169).

Resveratrol was first reported to protect mice from chemically induced skin cancers in 1997 (218). Since then, numerous papers have confirmed that resveratrol is a potent chemo-protective agent in mouse models of cancer. Interestingly, a recent paper by the McBurney laboratory (125) showed that resveratrol is significantly less protective against skin cancer in

the SIRT1 null mouse. Only 20% of the resveratrol-treated wild-type mice developed tumors after 15 weeks, whereas 75% of SIRT1 null mice developed tumors. Thus, some of the protective effects of resveratrol against skin cancer in this model are mediated by SIRT1.

Given the critical role of yeast Sir2 at telomeres, many researchers have speculated that SIRT1 may also be involved in telomere biology. SIRT1 has not been detected at telomeres in mammalian cells, but hematopoietic stem cells obtained from SIRT1-deficient mice show increased growth capacity, seemingly due to increased telomerase activity (219). In these studies, the overexpression or downregulation of SIRT1 alone had no effect on the life span of human diploid .broblasts, indicating that genetic or epigenetic alterations may occur during the development of the SIRT1 knockout mouse. Growth rates, however, were lower in the SIRT1-overexpressor lines (219).

Other Sirtuins in Cancer

Another sirtuin studied in the context of cancer is SIRT2, a tubulin deacetylase that is required for normal mitotic progression (17,180) and that controls mitotic checkpoint functions in early metaphase to prevent chromosomal instability (181,184). SIRT2 abundance increases dramatically during mitosis, and SIRT2 is multiply phosphorylated during the G(2)-to-M transition of the cell cycle. Cells stably over-expressing the wild-type SIRT2, but not missense mutants lacking SIRT2 activity, have a marked prolongation of the mitotic phase of the cell cycle (220). In glioma and glioma-derived cell lines, SIRT2 is downregulated, indicating that increased expression of SIRT2 may be beneficial in the disease (182).

Inhibition or downregulation of SIRT2 interferes with cell-cycle progression and can promote cell-cycle arrest in vitro (180), indicating that inhibition of SIRT2 may be useful in treating some cancers. A novel SIRT2 inhibitor, AC-93253, is selective for SIRT2 over SIRT1 and has cytotoxicity for four different tumor cell lines (221). A dual inhibitor of SIRT1 and -2, cambinol, was found to inhibit Burkitt lymphoma xenografts (see Figure 2) (222), but whether this effect occurs via SIRT1 or -2 or is an off-target effect remains to be determined. One possible clue comes from a recent paper, which found that both SIRT1 and -2 deacetylate H3K56 and that this acetylation marker is increased in multiple types of cancer (183). Clearly, more work is needed to assess whether any of these compounds could be effective cancer therapies in humans.

As for the other sirtuins, SIRT3–7, there are only a few hints that they may be important in cancer biology. SIRT3 can be proapoptotic in HCT116 cells via JNK2, a pathway independent from SIRT1 (223). In other situations, such as in response to DNA damage when NAD$^+$ levels in mitochondria fall below critical levels, SIRT3 (and SIRT4) can be antiapoptotic (44). GCIP (or CCNDBP1/DIP/HHM) is a potential tumor suppressor on chromosome 15 that is downregulated in colon, breast, and prostate cancers. GCIP specifically interacts with one of the class III HDAC proteins, SIRT6, which is important for maintaining genome stability (22,224). This suggests a possible function of GCIP-SIRT6 in tumor suppression (225).

In summary, as researchers have tested the function of SIRT1 in mouse models, they have failed to confirm in vitro data suggesting that *SIRT1* is a tumor-promoting gene. Of the various mouse strains that have been engineered to overexpress *SIRT1*—at least six have been reported —none has shown a predisposition for cancer, even in cancer-prone mouse models such as *p53*$^{+/-}$ and *APC*$^{min/+}$. The *SIRT1* null mice have also failed to provide compelling evidence for *SIRT1* as a tumor promoter, and in fact the mice are more prone to p53-induced cancers. Possible reasons for the discrepancy between the cell culture and in vivo data are that (*a*) the effects of SIRT1 are cell type specific and/or mouse model specific, (*b*) the in vivo role of SIRT1 is not to promote cell growth but to prevent premature senescence that leads to aging and disease, and (*c*) SIRT1 interacts with p53 to alter respiration or another mitochondrial

function (226,227). Only with greater numbers of studies of human tissue samples and animal models will we obtain a better sense of whether, and if so under what circumstances, SIRT1 can promote tumorigenesis and tumor growth in vivo.

CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD), the leading cause of death in the world, is a progressive, multistep process that depends on complex interactions among cholesterol biosynthesis, the immune system, and vascular endothelial cell function (228). Risk factors include higher-than-normal levels of oxidized low-density lipoprotein cholesterol, vascular injury, and increased inflammation and cholesterol deposition by macrophages infiltrating smooth muscle below the vascular endothelium. For many decades, CR has been known to protect from CVD in animal (85) and human studies (229), but whether these effects could be mediated by a single genetic pathway or be susceptible to a single pharmacological intervention is unclear.

Endothelial Function

The increasing evidence that CR may be mediated, at least in part, by sirtuins (230,231) led to speculation that sirtuins may be useful in preventing or treating CVD (5). A recent influx of data on SIRT1 in CVD has confirmed this view, making SIRT1 a promising molecular target for successful therapeutic intervention for CVD (232). SIRT1 is highly expressed in endothelial cells and controls functions critical to suppressing the development of atherosclerosis, including upregulation of endothelial nitric oxide synthase (eNOS), a reduction in cell senescence of smooth muscle cells, suppression of inflammation and ROS in arteries, and increased vascular growth (232,233). SIRT1 has also been shown to alter cholesterol biosynthesis in the liver and macrophages (143) and to reduce serum lipid levels (138). Together, these results are consistent with SIRT1 being a major player in the prevention of CVD by CR. As yet, there is only speculation about the potential role of SIRT2–7 in CVD.

Apart from SIRT1's link to CR in budding yeast, the first indication that this sirtuin may protect against CVD in mammals was the relationship between resveratrol and SIRT1. Since the early 1990s, resveratrol has been touted as the source of the cardioprotective benefits of red wine, and in cell culture and animal models resveratrol has been shown to be highly anti-inflammatory and protective against CVD (234). Until 2003, however, the mechanism of protection was considered to be entirely due to resveratrol's antioxidant properties. In 2006, resveratrol was shown to decrease ROS, inflammation, and apoptosis in the aortas of elderly mice on a high-fat diet while maintaining normal endothelial function (96). Although these effects were coincident with deacetylation of a SIRT1 target, PGC-1α, this study suggested that SIRT1 was involved in the effects of resveratrol. Since then, however, evidence has mounted that SIRT1 is directly responsible for these protective effects. One of the key requirements for the assembly and repair of damage to the cardiovascular system is angiogenesis. Potente and colleagues (197) showed that mice lacking SIRT1 in the endothelium appear normal from a development standpoint but have an impaired ability to form new blood vessels in response to ischemia. Through use of endothelial cell culture and time-lapse analysis of vessel formation in developing zebrafish embryos with fluorescently labeled endothelial cells, SIRT1 was identified as a requisite factor for endothelial sprouting and vessel navigation. Whether or not the lack of a developmental defect in the adult SIRT1 knockout mice is due to redundancy of sirtuins or a difference between mice and fish remains to be seen.

Vessel Inflammation

A major function of SIRT1 that protects the cardiovascular system is its ability to suppress inflammation. Isolated macrophages and endothelial cells treated with SIRT1 activators have lower levels of inflammatory cytokines, including TNFα, intercellular adhesion molecule

(ICAM)-1, interleukin (IL)-6, IL-1, and inducible NOS (iNOS) (121,235,236). A similar anti-inflammatory response to SIRT1 activation is seen in mice and rats exposed to a high-fat diet or to cigarette smoke (95,237,238). In cultured coronary arterial endothelial cells (CAECs), the protective effects of resveratrol were abolished by knockdown of SIRT1, whereas the overexpression of SIRT1 mimicked the effects of resveratrol (238).

Some of the cardiovascular benefits of CR and pharmacologic activation of SIRT1 seem to stem from SIRT1's ability to induce nitric oxide signaling. eNOS, an enzyme that generates nitric oxide, is atheroprotective and promotes blood vessel relaxation. Resveratrol has been shown to induce the expression of eNOS when tested either alone (205,239) or in combination with a statin (240), albeit in one rat study it failed to induce eNOS expression (241).

The first direct link between eNOS and SIRT1 was established upon the demonstration that SIRT1 physically interacts with eNOS and deacetylates lysines 496 and 506 in the calmodulin-binding domain of eNOS, leading to enhanced eNOS activity (242). Mice on a CR diet had lower levels of eNOS acetylation, consistent with the known increase in SIRT1 levels and eNOS in rodents on CR (88,243,244). *Nampt/PBEF*, a NAD$^+$ biosynthetic gene and activator of SIRT1, endows human endothelial cells with increased replicative life span and enhanced angiogenic capacity in a high-glucose environment (245). Conversely, inhibition of SIRT1 expression decreases nitric oxide bioavailability, inhibits endothelium-dependent vasorelaxation, and induces premature senescence of endothelial cells (242). In agreement with the in vitro data, transgenic overexpression of SIRT1 in endothelial cells prevents the loss of vasorelaxation and lowers the number of atherosclerotic plaques in the *apoE$^{-/-}$* model of atherogenesis (246), without affecting blood lipid or glucose levels (247). Interestingly, eNOS signaling is necessary for SIRT1 induction by CR (244), indicating that SIRT1-eNOS-SIRT1 signaling forms a positive feedback loop that amplifies the effects of CR and resveratrol (Figure 8).

Recently, Ungvari and colleagues (238) showed that resveratrol treatment attenuated the deleterious effects of cigarette smoke extract in rat arteries and CAECs. The inflammatory markers (ICAM-1, iNOS, IL-6, and TNFα) were considerably reduced, as were nuclear factor kappa B (NF-κB) activation and inflammatory gene expression. In CAECs, these protective effects, including the suppression of apoptosis, were prevented by knockdown of SIRT1, whereas the overexpression of SIRT1 mimicked the effects of resveratrol. Thus, the vasoprotective and anti-inflammatory effects of resveratrol require SIRT1, making this sirtuin a potential drug target for the treatment of CVD and vascular aging.

With regard to the other sirtuins, SIRT2–7, little is known about their potential for influencing the cardiovascular system. SIRT3 probably protects against the damage caused by myocardial infarction or chronic heart failure given that it is a stress- and exercise-responsive gene that protects cardiac myocytes from genotoxic and oxidative stress-mediated cell death. The nuclear form of SIRT3 is thought to promote cell survival by deacetylating Ku70, causing it to sequester the proapoptotic protein Bax away from mitochondria (163).

Vascularization

Another attribute of SIRT1 relevant to CVD is its ability to downregulate FOXO signaling. FOXO transcription family members are essential negative regulators of blood vessel formation, and FOXO1 is the primary mediator (248–250). SIRT1 targets FOXO factors for deacetylation, thus reducing FOXO1-dependent transcriptional activity (248). SIRT1 also interacts with a component of the Notch signaling pathway, the Hairy and Enhancer of split basic loop-helix-loop-helix repressor protein Hey2 (251). As Potente & Dimmeler (198) point out, given the role of Hey2 in vascular patterning in vertebrates, the interaction between SIRT1

and Hey2 indicates that SIRT1 may function downstream of Notch and modulate Hey2 endothelial angiogenic activity (198).

Cholesterol Metabolism

The key role of oxidized low-density lipoprotein cholesterol in the progression of atherosclerosis and the protective function of high-density lipoprotein are well known. Guarente and colleagues (143) showed that SIRT1 deacetylates and thereby activates the nuclear receptor LXR, promoting the transcription of LXR target genes involved in lipid metabolism, including the ABCA1 transporter, and in mediating the efflux of cholesterol from peripheral tissues. It is tempting to speculate that one of the long-term benefits of SIRT1 activation by CR or a SIRT1 activator could be the efflux of cholesterol from peripheral tissues to slow atherosclerotic plaque formation (198). In the liver, SIRT1 controls hepatic triglyceride synthesis by activating genes such as SREBP1. Consistent with this finding, SIRT1 knockout mice show reductions in plasma, hepatic triglyceride accumulation, and decreased high-density lipoprotein cholesterol but, surprisingly, increased glucose tolerance (143).

NEURODEGENERATION

Unlike the situation for CVD, the role of sirtuins in neuroprotection is not entirely clear. Although there are numerous papers showing that increases in NAD^+ and SIRT1 are neuroprotective (252,253), unresolved questions about SIRT1 remain. Is it always beneficial to activate SIRT1, or in some circumstances should it be inhibited? Why is the catalytic activity of SIRT1 not always required for neuroprotection? There is also debate about whether the neuroprotection provided by resveratrol and NAM are mediated via SIRT1, an alternate sirtuin, or an entirely different mechanism. One study suggested that SIRT1 is harmful to neurons (98). With regard to the other sirtuins (SIRT3–7), little is known apart from one report (254) that increased expression of either SIRT2, -3, or -6 promotes apoptosis in neurons exposed to low potassium and that SIRT6 is modestly protective against homocysteic acid toxicity in neuroblastoma cells. Two studies have reported cytoprotective functions for SIRT3 and -4 (44,163); the latter study showing that these enzymes are required for the protection of cells by increases in Nampt and mitochondrial NAD^+ (44). It will be interesting to test whether this protection can also be observed for neurons.

Wallerian Degeneration and Parkinson's Disease

By the early 2000s, SIRT1 was already known to suppress apoptosis in a variety of cell types (31,88,168,187,255), but it was the study by Milbrandt and colleagues (252) that provided the first evidence that SIRT1 is neuroprotective. Their study showed that SIRT1 was necessary for the protection of neurons against Wallerian degeneration (WD), which is essentially the die-back of axons following a nerve crush injury (252,256). Mutant mice that are protected from Wallerian degeneration (known as Wld^S mice) have greatly increased resistance to WD due to a mutation that amplifies the NAD^+ biosynthetic gene Nmnat fused to a ubiquitin assembly protein, Ufd2 (257,258). The Milbrandt group's paper (252) proposed that increased NAD^+ production by Nmnat and subsequent SIRT1 activation were necessary for neuroprotection. Although the ability of SIRT1 to provide neuroprotection in a variety of contexts is now undisputed, and although SIRT1 has been shown to require catalytic activity in human neurons, there continues to be debate about how Nmnat1 protects cells and whether SIRT1 is involved (259,260). For example, SIRT1 has been found to be dispensable for the protective effects of NAD^+, and there are conflicting data on whether Nmnat alone can protect neurons in vivo or whether catalytic activity is required (253,259,261–263). However, recent data in vivo support the Milbrandt model (253). A recent study showing that resveratrol abolishes the resistance of Wld^S mice to WD proposed that SIRT2 activation was responsible (264), although this result is confusing given that resveratrol does not activate SIRT2 in vitro

(94). This work also seems to run counter to a study showing that inhibition of SIRT1 provided neuroprotection in a mouse model of Parkinson's disease (265).

Although the role of SIRT1 and -2 in WD is unclear, SIRT1 provides neuroprotection in the context of a variety of cell stresses, both in vitro and in vivo. In 2004, Brunet et al. (187) were the first to show SIRT1-mediated neuroprotection using cerebellar granule neurons, which was traced to SIRT1's ability to target FOXO3 for deacetylation, thereby dampening FOXO-mediated cell death. In the same year, Sinclair and colleagues (62,88) showed that SIRT1 targets the C terminus of Ku70 for deacetylation, which promotes the binding of Bax to Ku70, thereby preventing Bax from initiating apoptosis.

Alzheimer's Disease and Amyotrophic Lateral Sclerosis

SIRT1 and the STACs are highly neuroprotective of neurons in vitro and in vivo. Tsai and colleagues (139) reported that SIRT1 protects neurons from apoptosis resulting from overexpression of mutant SOD1-G37R, an allele linked to amyotrophic lateral sclerosis. The authors also reported protection from the p25 mutant version of CDK5, a suspected contributor to axonopathies and Alzheimer's disease in humans. In a p25 transgenic mouse model of Alzheimer's disease and axonopathies, resveratrol and SIRT1 provided protection from neurodegeneration and decline in cognition (139). Human SIRT1 and resveratrol were also shown by the Pasinetti and Sauve groups (86) to reduce the A beta peptide content of neurons in the Swedish Tg2576 mouse model of Alzheimer's disease that expresses mutant human cDNA for APP. The same papers proposed that the neuroprotective effects of CR may be due to changes in the NAD^+/NAM ratio and subsequent SIRT1-mediated activation of ROCK1, a Rho kinase that induces the protective, non-amyloidogenic alpha-secretase pathway (86).

Optic Neuritis and Age-Related Macular Degeneration

Optic neuritis is inflammation of the optic nerve; it causes loss of vision, usually due to swelling and destruction of the myelin sheath covering the optic nerve, and is a precursor to multiple sclerosis. Shindler and colleagues (267) found that the activation of SIRT1 by intravitreal injection of resveratrol or a precursor to NAD^+, NR, significantly attenuated the loss of retinal ganglial cells and that this neuroprotective effect was blocked by sirtinol, a SIRT1 inhibitor. Another eye disease, age-related macular degeneration, is the leading cause of severe vision loss in the elderly. Polymorphisms in the *complement factor H*(*CFH*) gene that reduce activity of CFH increase the risk of age-related macular degeneration. Overexpression of SIRT1 has also been shown to attenuate FOXO3 recruitment to the regulatory region of the *CFH* gene and to reverse hydrogen peroxide–induced repression of *CFH* gene expression, raising the possibility that SIRT1 activation may ameliorate or slow the progression of age-related macular degeneration (268).

Neural Progenitors

A recent study showed that SIRT1 controls the fate of neural progenitor cells, which are thought to regenerate neurons to compensate for damage in the central nervous system. Many neurodegenerative diseases of the brain are characterized by the emergence of more astrocytes than neurons through a process known as reactive astrogliosis, which may be induced in part by the oxidative milieu caused by inflammation. A recent study found that activation of SIRT1 by resveratrol suppresses proliferation of neural progenitor cells and directs their differentiation toward the astroglial lineage rather than the neuronal lineage, mimicking the effect of oxidative conditions (269). The effect of resveratrol was blocked by siRNAs against SIRT1 mRNA. The mechanism of SIRT1 action appears to be the binding to Hes1 to deacetylate H3K9 and stabilizes the TLE1 corepressor complex that inhibits the proneuronal transcription factor Mash1. Another study concluded that SIRT1 represses the Notch1-Hes1 signaling pathway and that the transient translocation of SIRT1 into the nucleus may have a role in neural

progenitor cell differentiation (270). Thus, SIRT1 may link oxidative stress, inflammation, and metabolism to the differentiation of neural progenitors (271). In this context, an inhibitor of SIRT1 may be beneficial by increasing and skewing the differentiation of neural progenitor cells into neurons. Along the same lines, Longo and colleagues (98) reported that mice lacking SIRT1 have lower levels of oxidized proteins and lipids and that knockdown of SIRT1 provided resistance to oxidative stress. It remains to be determined, however, whether increased expression of SIRT1 has the opposite effect.

INFLAMMATION AND INFECTION

SIRT1 as a Modulator of Nuclear Factor Kappa B

Within the past few years, sirtuins have been identified as novel regulators of the immune system, and numerous studies show that SIRT1 can suppress inflammation in multiple tissues (93,121,235,236,238,276,277). One of the master regulators of both adaptive and innate immunity is the NF-κB system, which is composed of receptors and signaling molecules whose functions have been highly conserved throughout evolution. NF-κB is a pleiotropic transcription factor that functions as a cytoplasmic sensor of danger signals, including hypoxia, oxidative stress, and genotoxic stress. NF-κB forms complexes with a number of other proteins, including Rel family members (RelA/p65, c-Rel, and RelB) and the NF-κB components p50 and p52. In the basal state, NF-κB complexes are retained in the cytoplasm via binding to the inhibitory IκB family of proteins. Upon activation, IκB proteins are phosphorylated and targeted for ubiquitination and subsequent degradation, freeing NF-κB complexes to translocate into the nucleus and trigger expression of target genes that are largely proinflammatory. Upregulation of the NF-κB-binding domain is strongly associated with aging, whereas NF-κB activity is strongly diminished by CR (273). SIRT1 has recently emerged as a potent inhibitor of the NF-κB system, providing a mechanistic link between inflammation and aging (275). After translocation to the nucleus, the p65 subunit is acetylated by p300 to enhance DNA binding. SIRT1 binds and deacetylates RelA/p65, inhibiting the transcriptional activity of NF-κB (202).

Sirtuins may also activate NF-κB signaling by regulating other factors, such as FOXO proteins, which converge on insulin signaling and stress pathways and suppress NF-κB signaling. FOXO3a overexpression can inhibit the TNFα-induced activation of NF-κB, and FOXO3a can promote apoptosis by suppressing NF-κB activity (reviewed in Reference 283). Interestingly, several studies have reported that SIRT1 interacts with and deacetylates FOXO proteins in worms and in mammalian systems (118,187,285), indicating that there may be an additional layer of NF-κB signaling control.

Metabolic disorders are associated with a state of chronic inflammation and the release from adipocytes and macrophages of the pro-inflammatory cytokines TNF-alpha and IL-6, which appear to impair insulin action in peripheral tissues. Several animal studies indicate that the beneficial effect of SIRT1 on metabolic disorders is mediated in part by its ability to suppress inflammation in adipocytes and macrophages. For example, SIRT1-overexpressing and resveratrol treated mice have lower levels of lipid-induced hepatic inflammation when challenged by a high-fat diet (93,95). These animals have decreased NF-κB activity, which results in correlates with decreased expression of proinflammatory cytokines, such as TNFα and IL-6. Similarly, SIRT1 expression in macrophages is inversely correlated to inflammatory gene expression (252). Modulation of SIRT1 activity also affects lipid accumulation in adipocytes, which has an impact on the etiology of metabolic diseases such as obesity and TDM. Consistent with this evidence, liver-specific SIRT1 null mice show increased signs of inflammation and NF-κB signaling when fed a high-fat diet (134). In contrast to these findings, a recent study that used SIRT1 whole-body null mice demonstrated that these animals

accumulate immunoglobin complexes in the kidney and liver but do not show alterations in their immune responses (278).

Smoking-Induced Inflammation

Interaction between SIRT1 and RelA/p65 is diminished by cigarette smoke, which causes increased acetylation and activation of NF-κB proinflammatory responses in macrophages (279). As discussed above, resveratrol treatment attenuates the deleterious effects of cigarette smoke extract in rat arteries and CAECs, effects that are prevented by knockdown of SIRT1 (238). Interestingly, CAECs cultured in the presence of serum from normal-fed mice exhibited NF-κB activation and inflammatory gene expression. In contrast, treatment of CAECs with serum from CR mice showed far less ROS generation, NF-κB activation, and induction of inflammatory genes, changes that were diminished by siRNA knockdown of SIRT1. Thus, the antioxidant and anti-inflammatory effects of CR on the vasculature may be mediated by circulating factors via a SIRT1-dependent mechanism (272).

Along similar lines, the lungs of smokers and patients with chronic obstructive pulmonary disease (COPD) have decreased levels of nuclear SIRT1, as compared with nonsmokers (280,281). Intranasal treatment with a selective STAC, SRT2172, prevented the pulmonary neutrophilia and reduction in exercise tolerance observed in a mouse smoking model of COPD, ostensibly by blocking an increase of matrix metalloproteinase 9 (281). This work suggests that SIRT1 activation may be a useful therapeutic approach to treating chronic inflammatory diseases such as COPD.

Inflammatory Uveitis

Uveitis, or inflammation of the middle layer of the eye, is estimated to be responsible for approximately 10% of the blindness in the United States. Resveratrol pretreatment has been shown to provide significant and dose-dependent suppression of leukocyte adhesion to retinal vessels at sites of ocular inflammation in endotoxin-induced uveitis mice (282). Levels of MCP-1, ICAM-1, 8-OhdG, and NF-κB are significantly reduced by resveratrol administration, coincident with increased SIRT1 activity in retinal pigment epithelium chondroidal cells (282).

Viral Infections

There is some evidence that human immunodeficiency virus (HIV)-1 suppresses SIRT1 during viral infection. NF-κB signaling has been implicated in the hyperactive immune response that contributes to HIV-1 infectivity and replication (286). The Tat protein of HIV-1 is required for the transcriptional activation of HIV. SIRT1 has been directly linked to this pathway on several levels. First, Tat itself is a target for SIRT1 (287,288); SIRT1 and Tat form a complex and synergistically activate the HIV promoter. Deacetylation by SIRT1 recycles Tat to its un-acetylated form, allowing it to bind transactivation responsive RNA and transcriptional elongation factors (288). Next, Tat also seems to regulate SIRT1 activity. TAT directly binds to SIRT1, blocking its ability to deacetylate p65 (288). In doing so, Tat hyperactivates NF-κB target gene expression in wild-type cells, but not in SIRT1 null cells. Thus, SIRT1, Tat, and NF-κB act in a circuit, in which SIRT1 promotes Tat and Tat neutralizes the negative effect of SIRT1 on NF-κB signaling.

SIRT6 and the Immune Response

The only other sirtuin linked to the regulation of immune responses is SIRT6, which may be a prolongevity protein. SIRT6 null mice have severe developmental defects, display hypoglycemia, and have a premature aging phenotype (224). Recently, it was found that SIRT6 interacts with the RelA/p65 component of the NF-κB complex and is recruited to some

promoters of NF-κB target genes (289). SIRT6 deacetylates histone H3 lysine 9 at these promoters and represses the expression of NF-κB targets. Consistently, the expression of several NF-κB target genes was elevated in SIRT6 null mice. An independent study found that SIRT6 regulates TNF production (290). Strikingly, hyperactive NF-κB signaling may be responsible for the short life span and degenerative phenotype of SIRT6 null mice, as haploinsufficiency of RelA rescues the premature aging phenotype of SIRT6 knockout animals.

PERSPECTIVE

It was only 10 years ago that yeast *SIR2* was shown to extend life span. Since then, it has become clear that *SIR2* is only one example of a large and highly conserved family of enzymes that control key cellular processes, such as fuel adaptation to low-nutrient conditions and mitochondrial biogenesis and function, in settings that include DNA repair, neuronal survival, and the maintenance of a youthful pattern of gene expression, among others.

However, much remains to be elucidated about the biology of sirtuins. For example, differences in mouse data reveal a complicated scenario that brings to light the fact that there is still much unknown regarding SIRT1 function, even though many of this sirtuin's substrates have been identified. Some of the confusion in interpreting these studies comes from conflicting data about when SIRT1 is active. For example, is SIRT1 induced in liver by fasting or by CR? Are earlier studies on SIRT1 now more difficult to interpret given that we know SIRT1 and its regulator, Nampt, are key components of the circadian clock that vary over a 12-h cycle (51, 53,291,292)? NAD^+ is an important physiological regulator of sirtuins, even in mitochondria (44), yet it is still not clear how NAD^+ is synthesized in various cellular compartments. Is it generated from NAM or NR, or is it transported as NMN through the membranes?

Despite the rapid advances in sirtuin biology, there are still no tools for accurately measuring SIRT1 activity on a native substrate in vitro or for assessing sirtuin activity in vivo. Currently, investigators use sirtuin protein level, NAD^+ concentration, and the acetylation state of sirtuin substrates in vivo as readouts. When used in combination, these methods can generate a rough snapshot. However, this snapshot may not be entirely accurate because sirtuins have overlapping targets, as in the case of SIRT1–3 and FOXO proteins. Sirtuins' activities may also be regulated by posttranslational modification or by inhibitory proteins, as in the case of DBC1 for SIRT1 (27,62). Thus, the above considerations highlight a need for assays that can provide a quantitative readout for the specific activities of SIRT1–7.

A major outstanding question is whether SIRT1 activation is safe enough to be used as a therapy for human diseases such as diabetes or CVD. Clearly CR has some drawbacks, including decreased fertility and osteoporosis, but these negative effects have not yet been reported for SIRT1-overexpressing mice or pharmacological STACs given to mice or humans (94,96, 114,293). Now that STACs are in human clinical trials for multiple diseases, we should know the answers to some of these questions within the next few years.

Although there are significant hurdles to overcome, in contrast to 10 years ago when the mammalian sirtuins were first investigated, there is little doubt that studies of these enzymes have greatly increased our knowledge about the elegant mechanisms that coordinate energy intake with organismal health and survival. In the coming decades, we should know if it is possible to safely target the sirtuins to treat diseases of aging.

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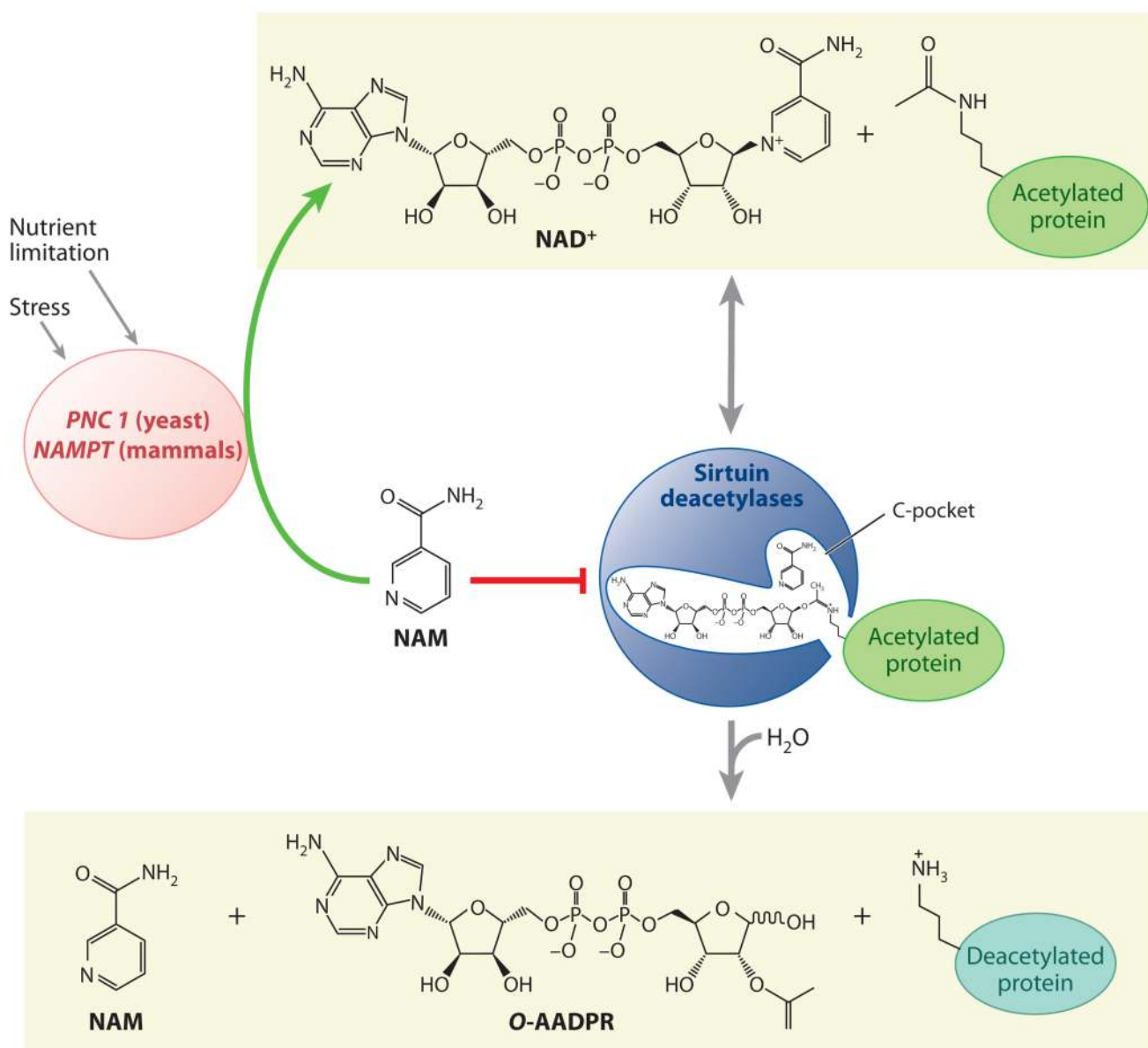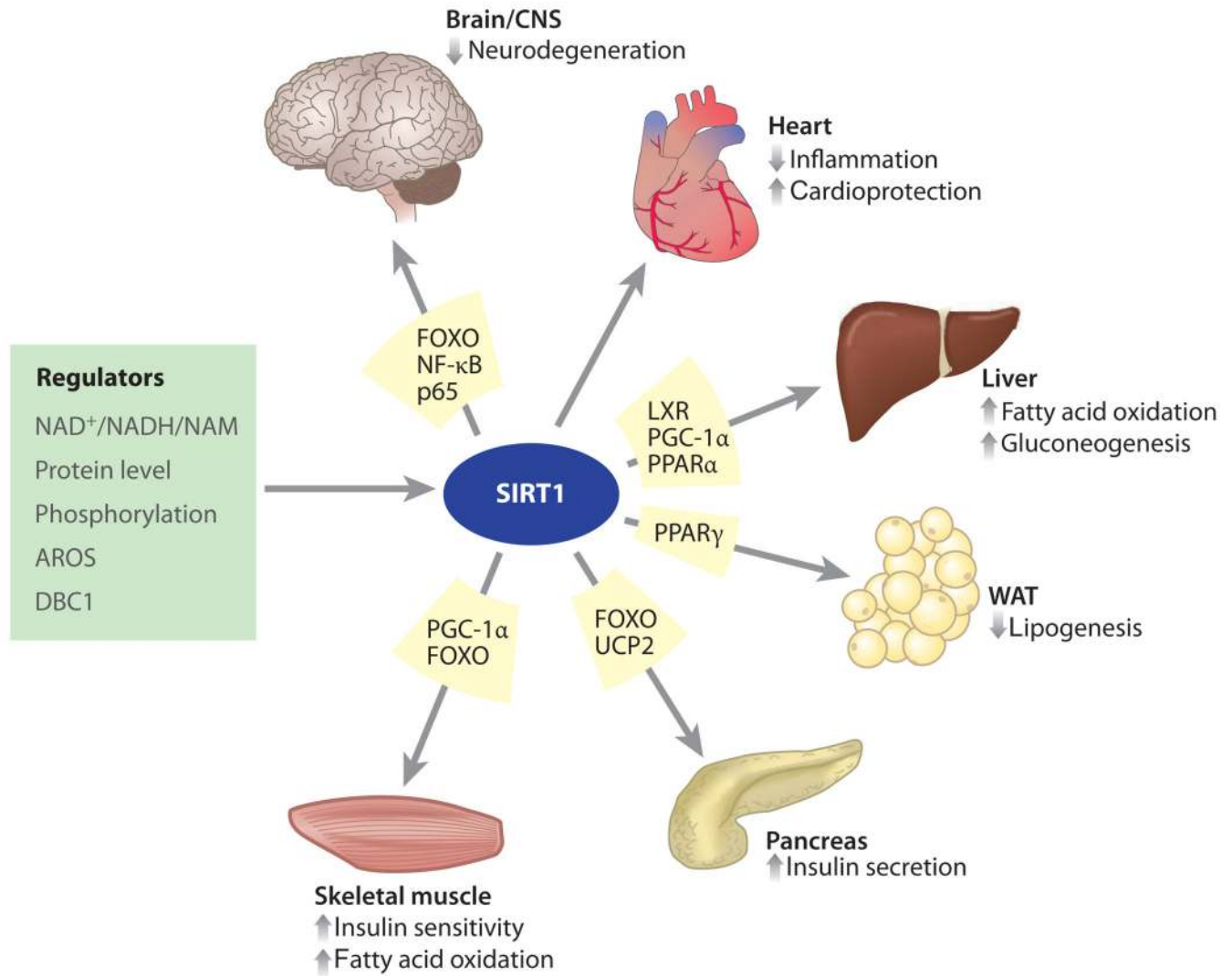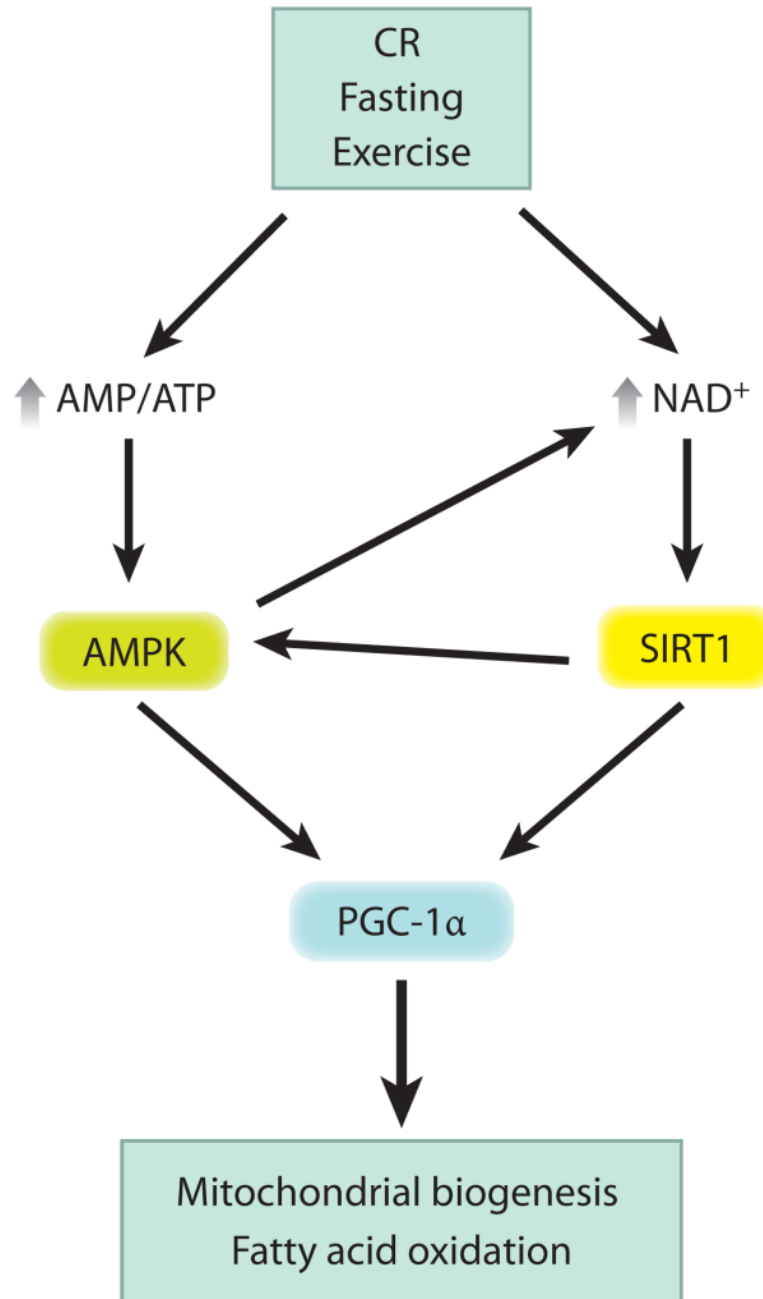
Figure 1.
The sirtuin deacetylation reaction and regulation by stress and nutrition. Unlike type I and II deacetylases, which hydrolyze the acetyl group on a substrate, sirtuin deacetylases catalyze an unprecedented two-step biological reaction that consumes nicotinamide adenine dinucleotide (NAD$^+$) and releases nicotinamide (NAM), O-acetyl-ADP-ribose (AADPR), and the deacetylated substrate. Amide-to-ester acyltransfer is unfavorable, but hydrolysis of NAD$^+$ can provide a favorable driving force for the overall sirtuin reaction. Evidence favors a mechanism in which electrophilic capture of the acetyl oxygen in an ADP-ribosyltransfer reaction forms an ADP-ribose peptide-imidate complex. This intermediate may last for a few seconds, enough time for NAM to enter the C-pocket and catalyze a reverse reaction. Activation of sirtuins can be facilitated by the removal of NAM and its conversion to NAD$^+$ by *PNC1* (yeast and simple metazoans) or *NAMPT* (mammals and alpha proteobacteria), two genes upregulated by stress and nutrient limitation.
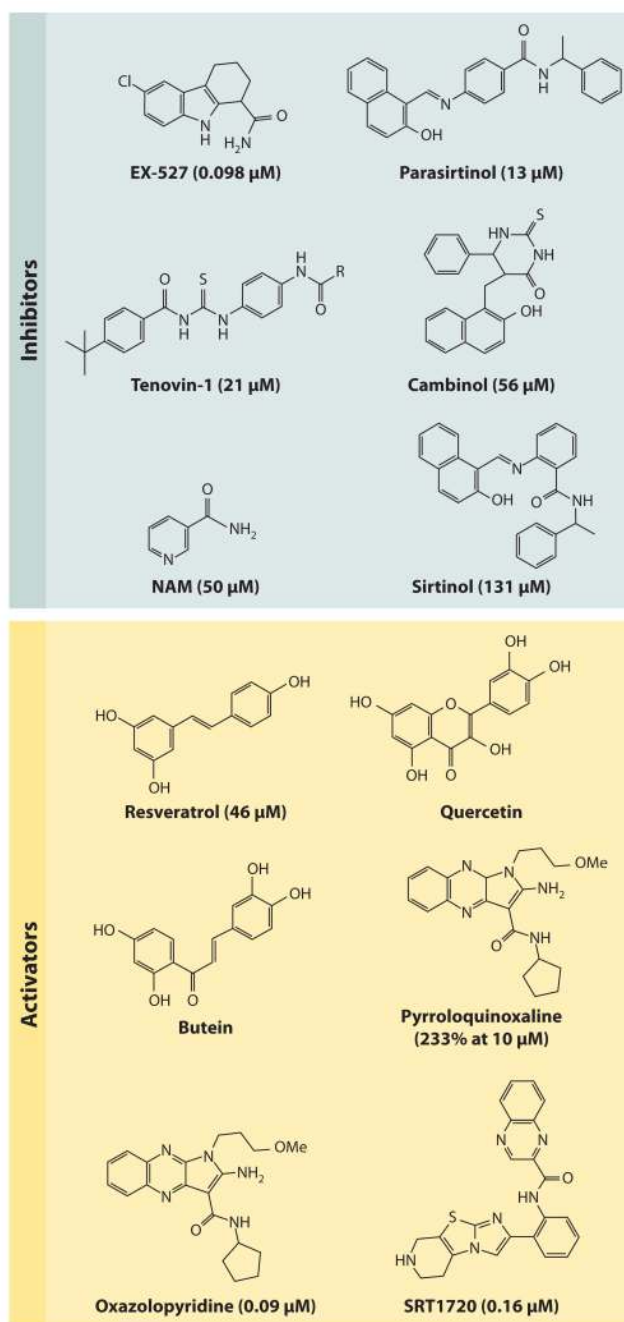
Figure 2.
Chemical inhibitors and activators of SIRT1. Over the past 10 years, a variety of small-molecule SIRT1-activating compounds (STACs) or inhibitors have been published. The known IC_{50}s and $EC_{1.5}$s for SIRT1 are shown in parentheses. Of all the inhibitors, only nicotinamide (NAM) is a physiological inhibitor, although analogs of NAM can activate sirtuins, apparently by occluding the C-pocket (see Figure 1). Polyphenolic activators such as resveratrol appear to bind the same site and activate via the same mechanism (i.e., primarily a lowering of the Michaelis constant effect) as that used by more potent activators such as SRT1720. The potency of inhibition is expressed as IC_{50} (the concentration to inhibit 50% activity), and activation is expressed as $EC_{1.5}$ (the concentration to activate 1.5-fold).
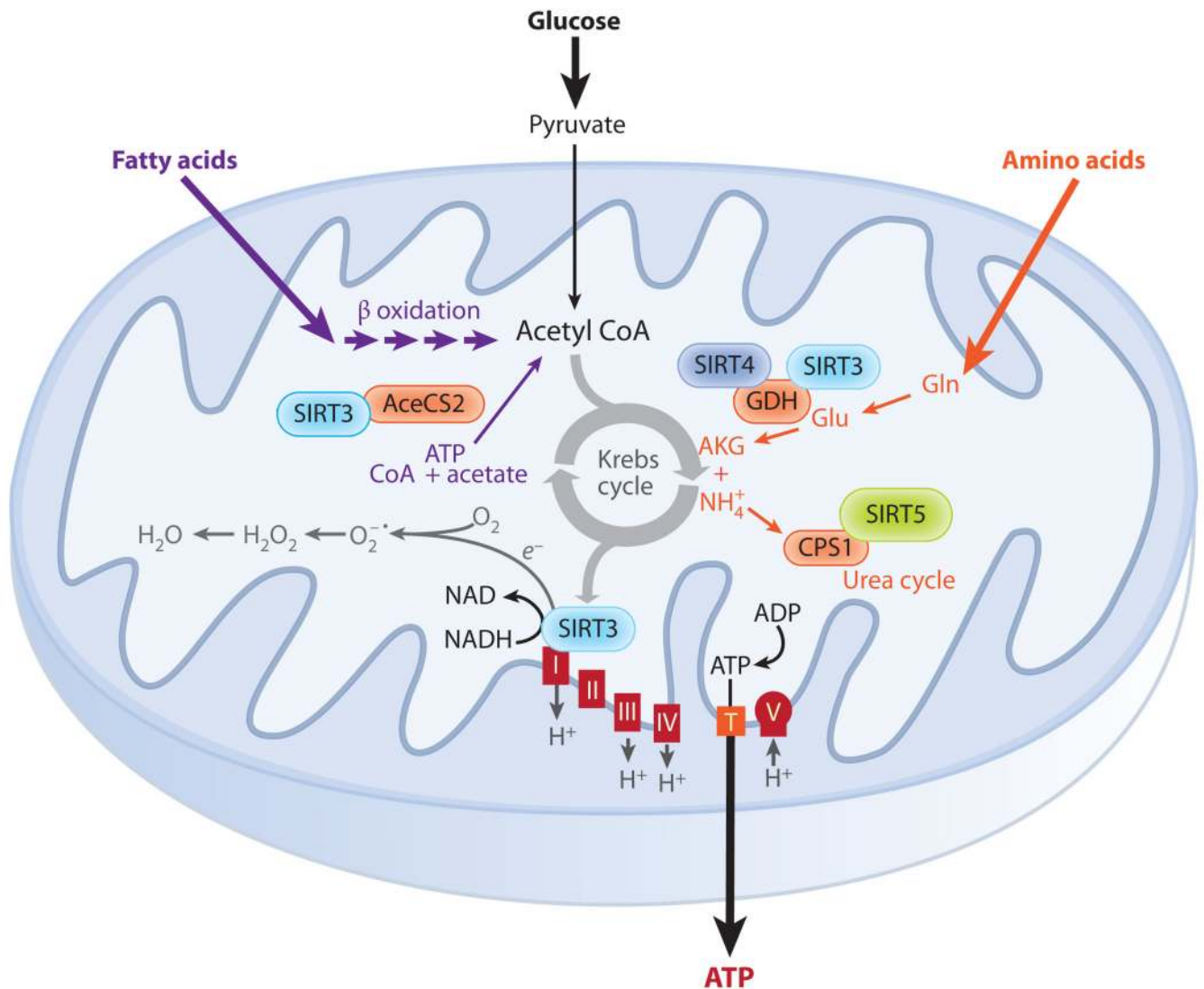
Figure 3.
SIRT1 regulation of age-related physiology. SIRT1 activity can be regulated through NAD+ (nicotinamide adenine dinucleotide) and nicotinamide (NAM) concentrations, by SIRT1 protein level, and by phosphorylation; SIRT1 can be activated by active regulator of SIRT1 (AROS) and inhibited by DBC1 (deleted in breast cancer 1). SIRT1 activation promotes survival of neurons and protects cardiomyocytes from death. In the liver, SIRT1 promotes fatty acid oxidation and gluconeogenesis during nutrient deprivation via LXR, PGC-1α, and PPARα. In white adipose tissue (WAT), SIRT1 decreases fat storage by repressing PPARγ. SIRT1 promotes insulin secretion and pancreatic beta cell survival by suppressing UCP2 and interacting with FOXO, respectively. In skeletal muscle, SIRT1 promotes mitochondrial biogenesis through the activation of PGC-1α. Abbreviations: CNS, central nervous system; FOXO, forkhead box transcription factor, subgroup O; LXR, liver X receptor; NF-κB, nuclear factor kappa B; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; PPARα, peroxisome proliferators-activated receptor alpha; UCP2, uncoupling protein 2. Adapted from Reference [15].
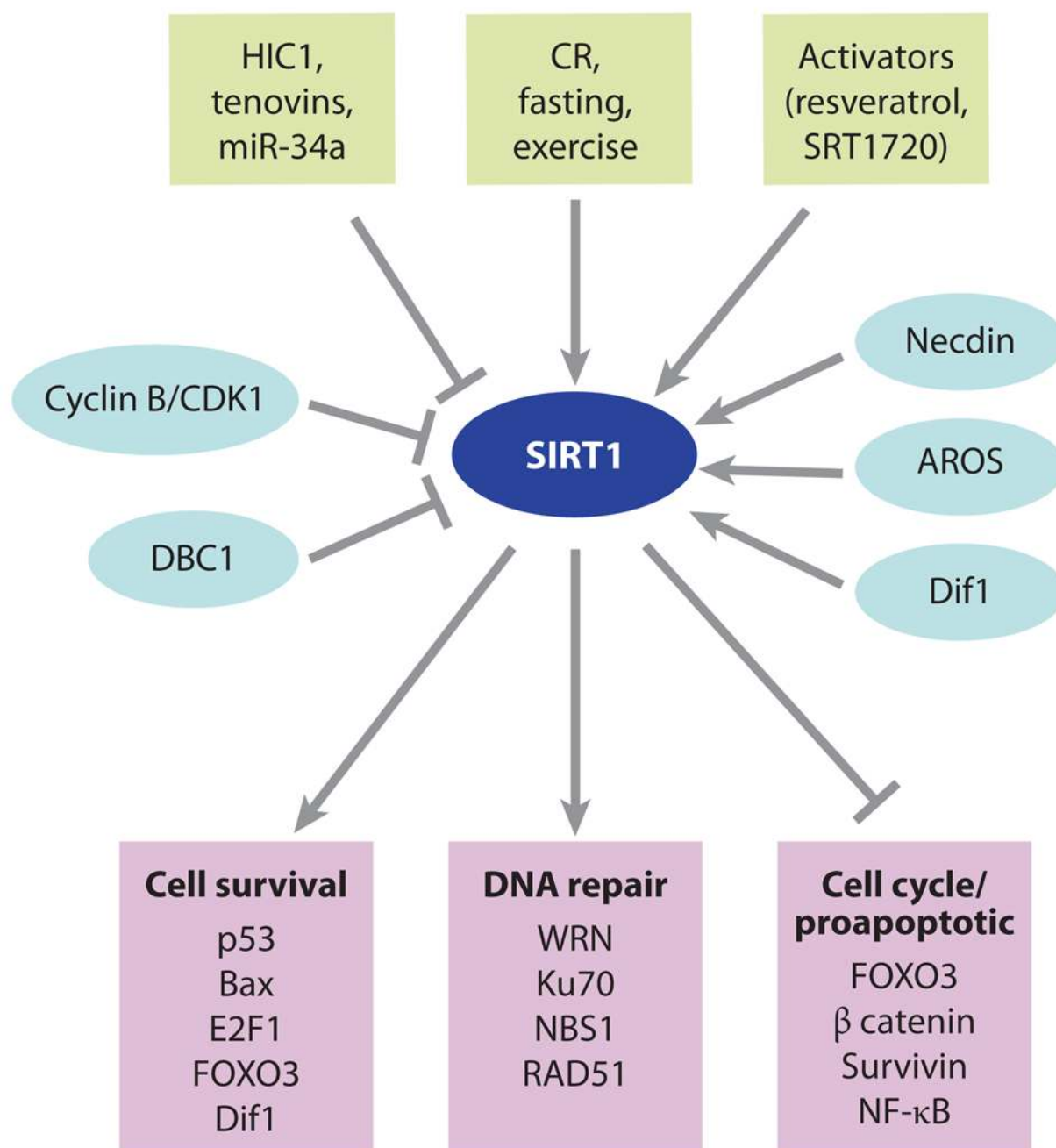
Figure 4.
The SIRT1-AMPK metabolic control network. Conditions of perceived energy deprivation, such as fasting, calorie restriction (CR), and exercise, increase the AMP/ATP ratio and activate AMP-activated protein kinase (AMPK). Energy deprivation also increases nicotinamide adenine dinucleotide (NAD^+) levels and activates the NAD^+-dependent deacetylase activity of SIRT1. Activated AMPK and SIRT1 converge by activating peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) via phosphorylation and deacetylation, respectively, to induce mitochondrial biogenesis and fatty acid oxidation. Cross talk in this pathway occurs because AMPK activity increases NAD^+, and SIRT1 also activates AMPK.
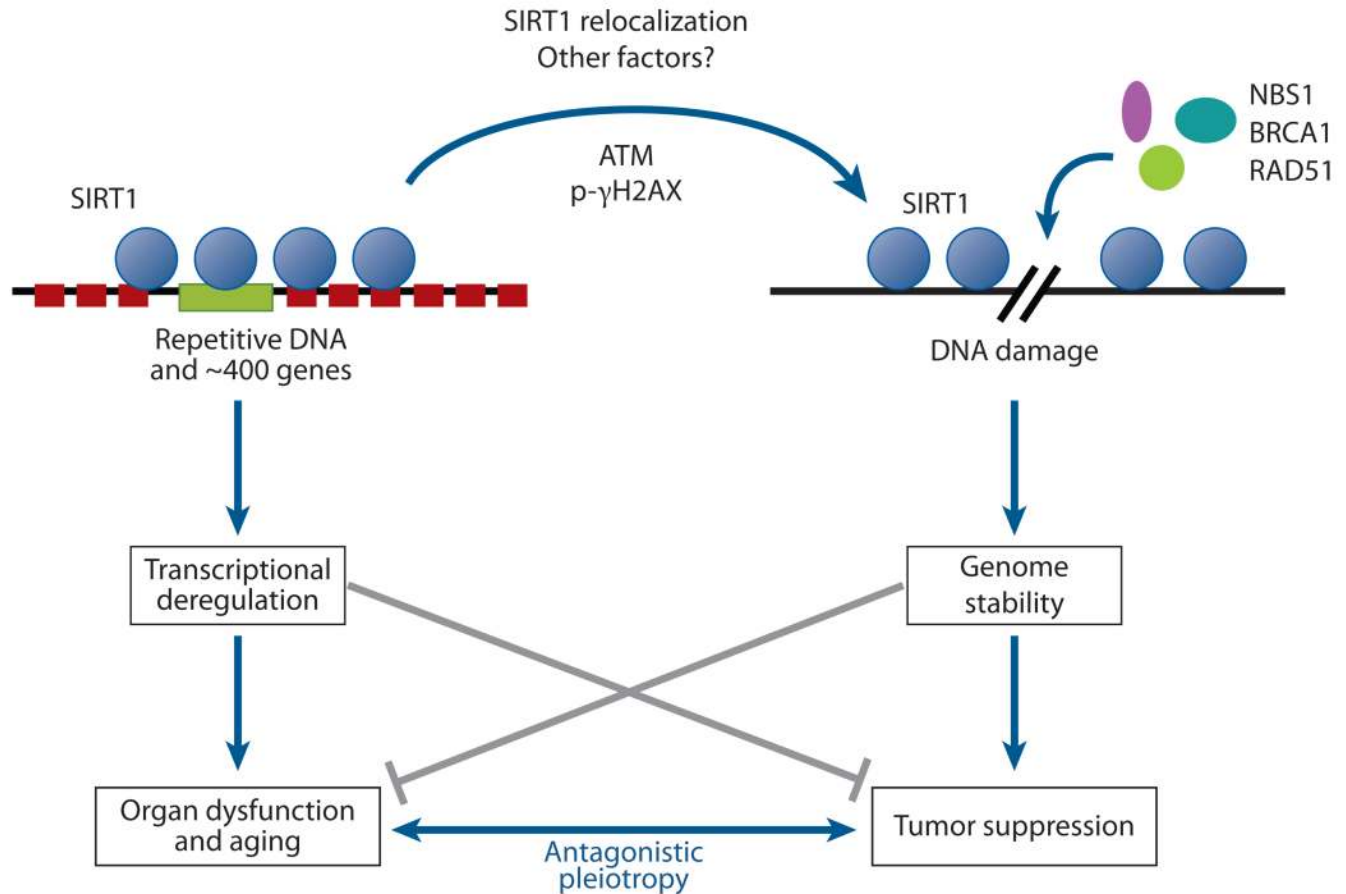
Figure 5.
Network of the mitochondrial sirtuins (SIRT3–5). Mitochondria can metabolize fuels, such as fatty acids, amino acids, and pyruvate, derived from glucose. Electrons pass through electron transport complexes (I–IV), generating a proton gradient that is used to drive ATP synthase to generate ATP. SIRT3 binds to complex I, regulating its activity and energy levels in the cell. SIRT3 also binds and deacetylates acetyl-CoA synthetase 2 (AceCS2) and glutamate dehydrogenase (GDH), activating their enzymatic activities. SIRT4 binds and represses GDH activity via ADP-ribosylation. SIRT5 deacetylates and activates carbamoyl phosphate synthetase 1 (CPS1), the rate-limiting step of the urea cycle.
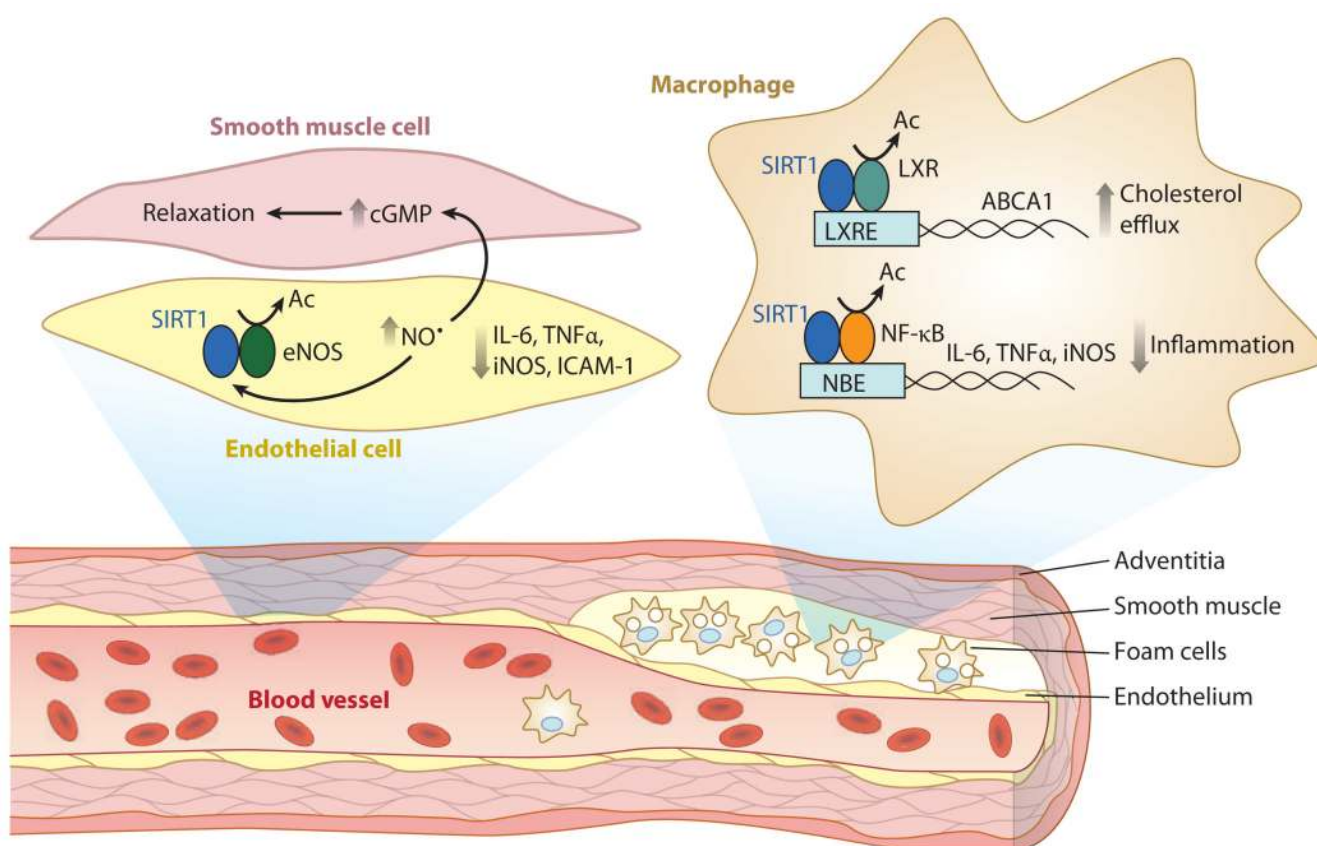
Figure 6.
SIRT1 plays key roles in cell survival and apoptosis. The complexity of SIRT1 regulation has made it a challenge to decipher SIRT1's role in cancer. The SIRT1 gene is under the control of both environmental stimuli such as fasting and exercise and microRNAs (miRNAs), tenovins, and the hypermethylated in cancer 1 (HIC1) transcriptional repressor. The SIRT1 enzyme can also be modulated by protein-protein interactions with deleted in breast cancer 1 (DBC1), adaptor response to oxidative stress (AROS), Dif1, and necdin. SIRT1 interacts with and deacetylates numerous proteins involved in cell survival, DNA repair, and apoptosis (Figure 3). On the basis of this information, it has been difficult to predict what SIRT1 overexpression or activation by SIRT1-activating compounds will do in vivo, but so far

experiments (171) point to SIRT1 acting as a tumor suppressor in the case of the *p53*$^{-/+}$, *breast cancer 1* (*BRCA1*), *7,12-dimethylbenz[α]anthracene* (*DMBA*), and *APC*$^{min+/-}$ models of lymphoma, breast, skin, and colon cancers, respectively. Abbreviations: CR, calorie restriction; DBC1, deleted in breast cancer 1; FOXO, forkhead box, subgroup O; HIC1, hypermethylated in cancer 1; NF-κB, nuclear factor kappa B.

Figure 7.
The relocalization of chromatin modifiers (RCM) hypothesis of aging stems from S. Imai and H. Kitano, who proposed in 1998 that changes in heterochromatin underlie the aging process. The idea was based in part on observations that, in response to DNA damage and aging, yeast SIR2 is released from silent loci and relocalized to DNA breaks, where it is hypothesized to organize chromatin to facilitate repair. During relocalization, expression of silent mating-type genes (*HML* and *HMR*) cause sterility, a hallmark of aging. Recent work shows that a similar process may drive aging in mammals. In response to DNA breaks or aging, SIRT1 also relocalizes away from open reading frames (ORFs) to DNA-break sites, seemingly to alter chromatin around the break site and recruit DNA damage–repair proteins such as RAD51 and NBS1. This relocalization of SIRT1, or the epigenetic changes it induces, is proposed to alter gene-expression patterns that result in tissue dysfunction and diseases associated with aging.

Figure 8.
The role of SIRT1 in protection from atherosclerosis and cardiovascular disease (CVD). In the normal progression of atherosclerosis, damage and inflammation of blood vessel walls promote the infiltration of macrophages, where they take up and accumulate oxidized low-density lipoprotein, becoming foam cells that can eventually rupture and promote additional inflammation and plaque formation, which in turn occludes blood flow. Overexpression of SIRT1 in endothelial cells or treatment of mice with resveratrol SIRT1 reduces reactive oxygen species in vessel walls and slows the progression of CVD, apparently through multiple mechanisms. These include the suppression of inflammation by increasing endothelial nitric oxide synthase (eNOS) and decreasing nuclear factor kappa B (NF-κB) activity. Increased retrograde cholesterol transport in macrophages may also be a contributing factor. Abbreviations: cGMP, cyclic guanosine monophosphate; ICAM, intercellular adhesion molecule 1; IL-6, interleukin-6; iNOS, inducible NO, nitric oxide synthase; TNFα, tumor necrosis factor alpha.

Table 1

Summary of the mammalian sirtuins

Sirtuin	Location	Interactions	Biology	Null phenotype
SIRT1	Nucleus	FOXO, PGC-1α NF-κB, Ku70, etc.	Metabolism, stress	Developmental defects, lethal in some backgrounds
SIRT2	Cytosol	Tubulin, H4, FOXO	Cell cycle	Developmentally normal
SIRT3	Mitochondria	AceCS2, GDH complex I	Thermogenesis, ATP production	Developmentally normal
SIRT4	Mitochondria	GDH, IDE, ANT	Insulin secretion	Developmentally normal
SIRT5	Mitochondria	CPS1	Urea cycle	Developmentally normal
SIRT6	Nucleus	Histone H3, NF-κB	Base excision repair, metabolism	Premature aging
SIRT7	Nucleolus	Pol I	rDNA transcription	Smaller size, short lifespan, heart defects

Abbreviations: AceCS2, acetyl-CoA-synthetase 2; ANT, adenide nucleotide translocator; CPS1, carbamoyl phosphate synthetase 1; FOXO, forkhead box, subgroup O; GDH, glutamate dehydrogenase; IDE, insulin degrading enzyme; NF-κB, nuclear factor kappa B; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; Pol I, DNA polymerase I; rDNA, recombinant DNA.