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The controversial world of sirtuins

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Abstract

The controversy around sirtuins and their functions in aging has drawn in the past few years as much attention, if not more, from the scientific community and the public as they did when first proposed as the key conserved aging regulators in eukaryotes. With some of the basic observations on sirtuin longevity promoting functions being questioned in popular model systems, researchers are wondering if this family of conserved enzymes still holds strong potential as therapeutic targets. This review examines the several controversial issues around sirtuins and their functions in aging, calorie restriction, as well as age-related diseases in light of recent studies in mammalian systems and discusses whether modulators of sirtuins still hold the secret of life.

Introduction

The word “sirtuin” was coined from its founding member Sir2 in budding yeast *Saccharomyces cerevisiae* [1]. SIR stands for Silent Information Regulator. Four SIR genes were identified through a genetic screen for mutations that suppressed transcription silencing [1]. Among them, *SIR2* is essential for silencing at all heterochromatin-like regions, including the ribosomal gene cluster (rDNA), telomeres, and the hidden mating type loci *HML/HMR*. The gene product Sir2 protein is an enzyme called histone deacetylase (HDAC, class III) that removes the acetyl group from acetylated lysines in histones. Very soon, many homologs of Sir2 were found from bacteria to humans, establishing a highly conserved class of enzymes [2].

Sirtuins are protein deacetylases and ADP-ribosyltransferases

Distinct from previously identified deacetylases (class I and II), sirtuins couple the deacetylation of lysine to the hydrolysis of NAD^+ by transferring the acetyl group to the ADP-ribose moiety to form O-acetyl-ADP-ribose, releasing free nicotinamide (Fig. 1A).

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Because NAD^+ is required for this reaction and the NAD^+/NADH is determined by the nutritional state of the cell, sirtuins directly link cellular metabolic signaling to the state of protein post-translational modifications. In addition to the NAD^+/NADH influence, this reaction is also regulated by one of its products, nicotinamide, which is a non-competitive inhibitor of sirtuins. The other reaction product, O-acetyl-ADP-ribose, has been suggested to facilitate heterochromatin formation and silencing, regulate ion channels, and modulate cellular redox state [3].

Some sirtuins are found to carry out reactions to remove larger PTM groups, such as malonyl and succinyl from their target proteins in a way very similar to deacetylation. Yet, other sirtuins do not exhibit the deacetylation activity, but rather act as ADP-ribosyltransferases, enzymes that add an ADP-ribosyl group to lysines [4] (Fig. 1B).

Sirtuins in simple model organisms

In budding yeast, Sir2 is recruited to silencing loci by other DNA binding factors such as Rap1 and removes acetyl group from histone H4 lysine 16 (H4K16), histone H3K56, and H3K4[5–7]. It forms a heterotrimeric complex with Sir3 and Sir4 at *HML/HMR* and telomeres, and exists in a homotrimeric form with Net1 and Cdc14 at rDNA [8]. Besides Sir2, there are four other sirtuins, Hst1, Hst2, Hst3, and Hst4, all of which are involved in transcription silencing [2], even though Hst2 predominantly localizes to the cytoplasm [9]. The involvement of sirtuins in aging was discovered through a genetic screen that identified a long-lived mutant featuring a C-terminal truncation to Sir4 [10]. It turns out that this mutation causes the Sir2-Sir3-Sir4 complex to relocalize from telomeres to the rDNA cluster in the nucleolus and to suppress the rDNA recombination and the formation of extrachromosomal rDNA circles (ERCs), an aging factor unique to yeast [11]. Deletion or inhibition of Sir2 shortens lifespan, whereas overexpression with a second integrated copy of the *SIR2* gene extends the replicative lifespan, suggesting that Sir2 promotes longevity and is a limiting factor for lifespan [12]. More recently, Sir2's function at telomeres is also shown to be important for longevity and that the Sir2 protein becomes much less abundant in old cells, providing more insights to the cause of yeast replicative aging [13].

In the nematode *Caenorhabditis elegans*, four sirtuins are identified with SIR-2.1 showing the highest similarity to yeast Sir2. Worm SIR-2.1 deacetylates H3K9 and is involved in transcription silencing and heterochromatin formation [14]. It is shown to interact with non-histone targets, 14-3-3 and DAF-16, to activate DAF-16 target genes, which is proposed to be a longevity promoting mechanism under stress [15]. Deletion of this gene moderately shortens lifespan [16]. However, the effect of *sir-2.1* overexpression has been recently disputed. The initially observed lifespan extension by either chromosomal duplication or transgene has been attributed to secondary mutations unrelated to *sir-2.1* [17,18].

In *Drosophila melanogaster*, five sirtuins have been found, with dSir2 being the closest homolog to yeast Sir2. dSir2 shows deacetylase activity *in vitro* and is required for heterochromatin silencing *in vivo* [19]. Knocking out dSir2 shortens lifespan [20]. Lifespan extension upon dSir2 overexpression has been found in a number of different configurations, including several different transgenes, different drivers, tissue-specific and adult-only

inductions [21]. However, several of these have also been challenged recently [18]. These controversies will be discussed in detail later.

Mammalian Sirtuins

In mammals, there are seven sirtuins, named SIRT1 to SIRT7. They are involved in a much broader range of cellular processes and pathways with distinct cellular localization and molecular targets (Table 1).

SIRT1 is the closest mammalian homolog of the yeast Sir2 protein in sequence and is the most studied mammalian sirtuin. SIRT1 predominantly localizes to the nucleus and acts as a deacetylase for histones H1K26 and H4K16 [22], as well as many non-histone targets (Table 1). Deacetylation of p53 by SIRT1 upon DNA damage and oxidative stress results in reduced apoptosis [23,24]. SIRT1 activates PGC1α through deacetylation and regulates mitochondria biogenesis and activities [25]. Acetylation state of FOXO transcription factors, regulated by SIRT1, is thought to selectively direct them to certain targets, representing another level of regulation of metabolism and stress response [4]. Upon inhibition of insulin signaling, SIRT1 is actively shuttled out of the nucleus into the cytoplasm. Recently, more evidence suggested SIRT1 is very profoundly involved in cancers and apoptosis, which is reviewed in greater detail below. Other newly identified novel functions of SIRT1 include neuroprotection against various neurodegenerative diseases [26,27], promoting liver function and regeneration [28], stem cell differentiation and cell fate determination [29–31], and delaying replicative senescence in primary fibroblasts [32].

SIRT2 exists in the cytoplasm, deacetylates tubulin, and regulates skeletal muscle differentiation [4]. Recently, SIRT2 has been found to accumulate in neurons in the central nervous system in aging brains and its microtubule deacetylase activity is linked to the pathology of brain aging and neurodegenerative diseases [33]. In the peripheral nervous system, however, SIRT2 appears important for nerve myelination and regeneration by deacetylating Par-3, a critical regulator of cell polarity and myelin assembly in Schwann cells [34]. In adipocytes, SIRT2 regulates metabolism through deacetylating FOXO1 and PCG1α [4,35]. When FOXO3 is deacetylated by SIRT1 or SIRT2, it is targeted for polyubiquitination and degradation [36]. Tissues from human breast cancers and hepatocellular carcinoma exhibit reduced levels of SIRT2, which regulates the anaphase-promoting complex (APC) activity in these tissues by deacetylating CDH1 and CDC20 to preserve genome integrity and to antagonize tumorigenesis [37].

SIRT3, SIRT4, and SIRT5 localize primarily to mitochondria. SIRT3 is the major mitochondrial deacetylase, targeting numerous enzymes playing critical roles in maintaining metabolic homeostasis [38]. In the fatty acid oxidation pathway, SIRT3 deacetylates the long-chain acyl CoA dehydrogenase (LCAD). Among enzymes involved in the TCA cycle, both isocitrate dehydrogenase (IDH2) and glutamate dehydrogenase (GDH) are deacetylation targets of SIRT3. Furthermore, SIRT3 deacetylates components in each step of the electron transport chain in oxidative phosphorylation [39]. The important mitochondrial enzyme required for generating acetyl-coA, AceCS2 is also a deacetylation target of SIRT3 [38]. In addition to these metabolic enzymes, SIRT3 provides protection against oxidative stress by

deacetylation and activation of SOD2, an important mitochondrial antioxidant enzyme [38]. SIRT4 is primarily an ADP-ribosylase targeting GDH [4]. More recently, SIRT4 has been shown to negatively regulate fatty acid oxidation in liver and muscle cells, an apparent opposing role to SIRT3 [4]. SIRT5 targets carbamoyl phosphate synthetase (CPS1) to remove malonyl or succinyl groups in a fashion very similar to deacetylation [4].

SIRT6 localizes in the nucleus and deacetylates histone H3K9 and H3K56 to maintain genome stability and telomere function [40]. More recently, SIRT6 is also found to be an ADP-ribosylase for PARP1 under oxidative stress and stimulates PARP1 function to support DNA repair [41].

SIRT7 exists in nucleolus and activates RNA polymerase I transcription [4]. Although several of its interaction partners have been identified, including RNA polI, rDNA transcription factor UBF, and chromatin remodeling complex WICH (consisting of WSTF and SNF2h) [42], SIRT7's catalytic activity and targets remain elusive.

Controversy 1: Sirtuins mediates effects of calorie restriction

Calorie restriction (CR) is the most robust way to extend lifespan in most model organisms studied so far, from yeast to primates. Yet, its molecular mechanism remains elusive [43]. After the discovery of sirtuins as a class of conserved aging regulators, genetic studies in simple organisms, such as yeast and fruit fly, suggested sirtuins mediates the effects of CR [44]. Specifically, in yeast, CR increases NAD^+/NADH ratio through elevated level of respiration. Since the Sir2 deacetylation reaction consumes NAD^+, an increased NAD^+/NADH ratio would favor the reaction. Meanwhile, CR enhances the salvage pathway of NAD^+ synthesis by up-regulating nicotinamidase Pnc1, an enzyme that converts nicotinamide to niacin (Fig. 2). Since nicotinamide is a product of the NAD^+-dependent deacetylation reaction and an inhibitor of sirtuins, activation of the salvage pathway under CR is thought to be another piece of important evidence supporting the activation of sirtuin under CR [44]. Most interesting evidence came from genetic experiments, where deletion of *SIR2* gene in yeast was reported to block the lifespan extension by CR. Similar results were also found in worms [45] and fruit flies [46]. In mammals, however, direct evidence for sirtuin's function in CR in other model systems remains to be evaluated. Nevertheless, in several cases, CR associated with increased sirtuin levels or activities in various mouse tissues, suggesting that sirtuins are involved in CR [47,48].

However, sirtuin's critical role in CR in yeast, one of the best defined genetic systems, has been challenged. These results suggested that yeast Sir2 is not required for CR to function in yeast as deletion of *SIR2* does not block the lifespan extension effect of CR when generation of extrachromosomal RNA circles (ERCs) was prevented through the *FOB1* deletion [49]. Moreover, CR still extended lifespan when *SIR2* and two of its paralogs *HST1* and *HST2* had all been deleted under such a condition [50]. Meanwhile, the TOR nutrient sensing kinase signaling pathway emerged as a conserved mechanism behind CR since disruption of *TOR1* mimics and is epistatic to CR [51]. Consistently, Sir2 is also not required for the lifespan extension by *TOR1* deletion, a proposed CR mimetic. Others have also found that the effect Sir2 activation, monitored by gene silencing, upon CR is moderate or undetectable, much

less than previous suggested [52]. In worms, it is also controversial: one study found *sir-2.1* required for CR mediated longevity [45]; while others showed *sir-2.1* to be dispensable for CR effects [53,54]. These studies have casted serious doubt on the fundamentals of sirtuin's role in CR.

Many questions remain unanswered amongst the debates around this controversy. In yeast, it seems clear that Sir2 is not required for CR when ERCs are blocked. However, Sir2 activity is activated by the metabolic (NAD^+/NADH ratio) changes associated with CR conditions. Since Sir2 promotes longevity as overexpression of SIR2 gene reproducibly extends lifespan in yeast, does activated Sir2 contribute to the longevity effect of CR? Interestingly, although sirtuin's role in CR has been challenged in yeast and worms, the evidence for Sir2 being critical for CR in flies seem plentiful [21]. In mammals, although direct genetic tests remain difficult, accumulating evidence suggest that sirtuins are regulated in various tissues at both expression levels and metabolic states. The results from changes in sirtuin abundance and activity can be at multitudes of levels, given the broad substrates of SIRT1 and mitochondria-specific sirtuins [47]. With the intimate regulatory relationship between sirtuins and various metabolic pathways regulated by AMPK, FOXO, and AceCS2, etc, it is an intriguing hypothesis that sirtuins mediate at least some of the effects of CR in mammals. However, the exact contributions of sirtuins to animal health, fitness, and potentially longevity under CR conditions remain to be explored.

A model could at least partially reconcile the controversy about sirtuins' role in CR. That is the activation of sirtuins, either through increase NAD^+/NADH ration or increased expression level, is one of the many anti-aging mechanisms exploited by CR (Fig. 3). Since it is a pathway further downstream of nutrient sensing and is not a common pathway node shared by other CR mechanisms, disruption of it does not block the majority effect of CR. Although sirtuins are not the central controllers of the longevity effects of CR, accumulating evidence does suggest their contributions to the full benefits of CR [4,55].

Controversy 2: Resveratrol is a sirtuin activator

Soon after sirtuins emerged as a class of conserved aging regulators, efforts were made to identify chemical inhibitors and potential activators of these enzymes. It was especially intriguing to search for activators since overexpression of sirtuins was shown to have longevity benefits. A screen using an *in vitro* deacetylation assay featuring a fluorophore labeled acetylated p53 peptide identified a number of inhibitors and activator for mammalian SIRT1. Among them, a compound found most enriched in red wines, resveratrol, activated the reaction as much as 13 fold by increasing the substrate binding affinity [56], and was proposed to mimic the calorie restriction *in vivo* [57]. Resveratrol extends lifespan for several model organisms, including yeast, worms, flies, and fish [58]. In mice, although the lifespan of mice fed on regular diet was not affected by resveratrol, it showed significant longevity and overall health benefits for mice fed on a high fat diet [59].

However, activation of sirtuins by resveratrol and other similar activators was quickly disputed because the covalently attached fluorophore was required for the enhanced substrate binding and stimulated deacetylation by SIRT1 [60–62] and the activation of sirtuins

cannot be observed *in vivo* [62]. The lifespan extension effect of resveratrol in worms and flies also seems dependent on strain backgrounds [63].

As the controversy unfolds, the interests in resveratrol did not subside, because many *in vivo* experiments continue to show its CR mimetic effects [64], as well as age related health benefits. These benefits include protection against cancer, cardiovascular diseases, insulin resistance, and cognitive problems [65]. Meanwhile, many reports showed that resveratrol mimics the effects of SIRT1 overexpression in various mammalian cell cultures [64]; many metabolic and signaling pathways activated by resveratrol also seemed to be dependent on the presence of sirtuins in various cell lines, tissues and mouse models [58]. Most interestingly, several clinic trials have shown encouraging results for resveratrol: reduced oxidative stress and inflammation; improved cardiovascular function; enhanced cognitive function; inhibition of cancerous growth; decreased insulin resistance in diabetic patients; and improved metabolic profiles for obese and aged individuals [66].

Are sirtuins the critical targets of resveratrol for all these health benefits? The exact molecular mechanism remains to be explored. In some cases, the answer might be “yes”; in others, the effect is likely mediated by other pathways. After all, resveratrol can activate AMPK through an upstream kinase LKB1 in the absence of SIRT1 [58]. Direct inhibition of p300 activity can be another mechanism independent of sirtuins [67]. For the situations where sirtuins do seem to mediate the health benefits, they may not be the direct targets of resveratrol, but rather being in the downstream pathways. To reconcile some apparent sirtuin-dependent resveratrol effects and the requirement of the artificial fluorophore for resveratrol stimulated sirtuin activation in biochemical assays, it is hypothesized that fluorophore labeled peptides are a closer mimic of the *in vivo* substrates of sirtuins because of their hydrophobicity [58]. However, biochemical assays with the full length SIRT1 substrate AceCS1 didn't support this hypothesis [61]. Obviously, the debate will continue until a satisfying molecular mechanism is identified.

Controversy 3: Overexpression of sirtuins extends lifespan

Extension of lifespan by overexpression of sirtuins was first reported for yeast Sir2 [12]. This overexpression was achieved by inserting another copy of the *SIR2* gene in the genome. This *SIR2* double copy strain showed a lifespan 30% greater than the wild-type control. Soon, similar effects were also shown in worms and flies.

In worms, the results came from a screen for long-lived mutants in strains containing partial genome duplications [68]. The strain with a genome duplication carrying *sir-2.1* extended lifespan by 10%; while two other strains with a duplication of the nearby regions excluding *sir-2.1* shortened lifespan. Three independent *sir-2.1* extrachromosomal transgenic lines showed lifespan extension by 20-50%. Lastly, three *sir-2.1* integrated strains also showed similar extensions. It is further suggested that SIR-2.1 functions in the same DAF-2 and DAF-16 regulated insulin signaling pathway. Later, an integrated low-copy *sir-2.1* transgenic strain also showed a lifespan extension of 26% [69].

In flies, three independent strains were constructed to express a GAL4 transcription activator driver and to carry the native dSir2 gene with a GAL4 binding site. They had an over 4 fold

increase in dSir2 expression and showed a lifespan extension of up to 57% [46]. This lifespan extension appeared to be dose-dependent, since a 10% dSir2 increase did not extend lifespan. Increase in lifespan was also observed when dSir2 was overexpressed in neurons, the type of cells normally having the highest dSir2 expression in adult flies.

Interestingly, the results for both worms and flies have been disputed by a study conducted among several labs in Europe and in the US [18]. First, they examined one of the three worm strains carrying an integrated *sir-2.1* transgene. Although it was long-lived compared to the wild-type, outcrossing 5 times to the wild-type strains abrogated the longevity effect. It was then found that a secondary mutation was responsible for the increase lifespan in the original *sir-2.1* transgenic strain. Then, a similar experiment was repeated for the integrated low-copy strain. After outcrossing 6 times, the mutant strain again failed to show lifespan extension. Lastly, upon *sir-2.1* RNAi knockdown, the original long-lived genome duplication strain still lived long, suggesting the longevity was not due to the increased *sir-2.1* expression. In flies, outcross of the long-lived dSir2 overexpression strain was still long-lived compared to the wild-type control, but the GAL4 driver only strain (no dSir2 overexpression) also had a similar long lifespan. Therefore, the longevity effect of the strain did not appear to be conferred by dSir2 overexpression, but rather the GAL4 driver itself. An independently created strain with a higher dSir2 overexpression level did not extend lifespan.

The debate quickly heated up. The original authors of the worm work responded by admitting the existence of the secondary mutation in one of the original long-lived *sir-2.1* transgenic strains, but maintained that their own outcrossing (6-8 times) resulted in strains still 10-15% longer-lived than the control strains [17]. In addition, another research group also confirmed that the lifespan extension of the integrated low-copy *sir-2.1* transgenic strain can be abrogated by RNAi knockdown of *sir-2.1* expression [70].

Debates on the longevity effect of sirtuin overexpression in these two important model systems are likely to continue until all the discrepancies are resolved. Better communication and prompt exchange of strains and protocols may be helpful in eliminating some technical disparities that might have contributed to the apparent discrepancy in results. The overexpression dosage of sirtuins may be an important factor to determine lifespan; hence it is prudent to compare a series of strains with different expression levels of sirtuins in lifespan experiments. The neuronal-specific dSir2 overexpression strain should also be outcrossed and evaluated the same way.

While the sirtuin's lifespan extension effect is disputed, works in mice have shown promising progress. Overexpression of the SIRT6 transgene extended lifespan for male mice, but not female mice [71]. The longevity effect appeared to be mediated by the insulin-like growth factor 1 (IGF1) signaling pathway, a mechanism consistent with findings in the previous worm studies. Yet, much more work remains to address whether activation of sirtuins can directly provide longevity benefits. Many new results in mice suggest a still promising future.

Controversy 4: Sirtuins are oncogenes or tumor suppressors

Not surprisingly, in mammals, many sirtuins targets are involved in cancer. SIRT1's pro-survival effects seem to promote oncogenesis and malignancy. It inactivates the p53 tumor suppressor through deacetylation and inhibits p53-dependent apoptosis upon DNA damage and oxidative stress [23,24]. SIRT1 interacts with E2F1, a cell cycle regulator, and interferes with its apoptotic function during DNA damage response [72]. DBC1 (Deleted in Breast Cancer 1), a tumor suppressor, binds to SIRT1 and inhibits its activity [73]. In many types of cancers, SIRT1 is found to be overexpressed [74]; knocking down SIRT1 in these cancer cells restores radiation induced apoptosis [75]. SIRT2 is up-regulated in acute myeloid leukemia (AML), deacetylating and activating Akt [76]. These observations and many others seem to support SIRT1 being an oncogene.

However, SIRT1 also clearly shows functions of a tumor suppressor in many other situations. Overexpression of SIRT1 in intestines reduces the incidence of colon cancer by deacetylation of oncogene β-catenin, preventing its localization to the nucleus [73]. SIRT1 also functions in DNA damage repair as it relocates to sites of DNA damage and promotes genome integrity [77]. Reduced SIRT1 expression levels associate with increase sarcoma and lymphoma in p53 heterozygous mice [78]. SIRT2 targets the APC to regulate cell cycle. Mice with disrupted SIRT2 develop cancers [37]. Interestingly, SIRT1 and SIRT2 levels are reduced in some types of cancers [73]. SIRT6 maintains telomere and genome stability [79] and its overexpression induces apoptosis in cancer cells [80].

How can sirtuins function as both oncogenes and tumor suppressors? At first, the situation seems extremely confusing, and sirtuin's functions in cancer appear dependent on the type of tissue or cancer. However, it does seem that their tumor suppressing roles are consistent with their anti-aging functions in animals as well as various tissues. It is likely that certain types of cancers have evolved a mechanism to usurp the general pro-survival properties of sirtuins in order to circumvent apoptosis. It remains to be investigated how such a mechanism works and if the tumor suppressor functions can be restored in such a case.

Despite controversies, can sirtuins be therapeutic targets in future medicine?

The answer is most likely affirmative for most researchers studying sirtuins and their functions. This is because, despite so many controversies, mammalian sirtuins have been clearly shown as a class of critical factors regulating many cellular processes, playing important functions in diverse tissues and systems. Sirtuin functions have been described in the central/peripheral nerve system, cardiovascular system, immune system, liver, bone, skeletal muscles, stem cells, and tissue regeneration. They have also been associated with most major diseases, such as cardiovascular diseases, cancer, metabolic disorders, neurodegenerative diseases, arthritis, and osteoporosis, all of which are age-related. For instance, in several types of cancers, knocking down SIRT1 sensitizes cancer cells to radiation and chemotherapies [73,81]. However, the complexity of activities of sirtuins, and their widespread roles and activities, increases the difficulties in determining how best to modulate them therapeutically.

Sirtuins are a class of epigenetic regulators that modulate the activity of their targets by removing covalently attached acetyl groups. Small molecule regulators targeting sirtuins would provide a robust, rapid, and yet reversible cellular response. Indeed, two HDAC inhibitor drugs have already been approved to treat a certain type of lymphoma, and many more are under clinical trials for several types of cancers.

It should be noted that current HDAC inhibitor drugs all target the class I and II HDACs, not sirtuins, the class III HDAC. Nevertheless, thanks to the interest in screening for small molecular modulators for sirtuins, many sirtuin-specific inhibitors have been discovered and characterized, several of which have been tested to treat cancer in mouse models [81]. Although resveratrol's function as a sirtuin activator is under debate, the compound rising with the fame of sirtuins did show many health benefits in clinical trials [66].

Current controversies around sirtuins will eventually lead us to a much better understanding for this class of enzymes, which should provide a clearer perspective for their use as targets in future medicine.

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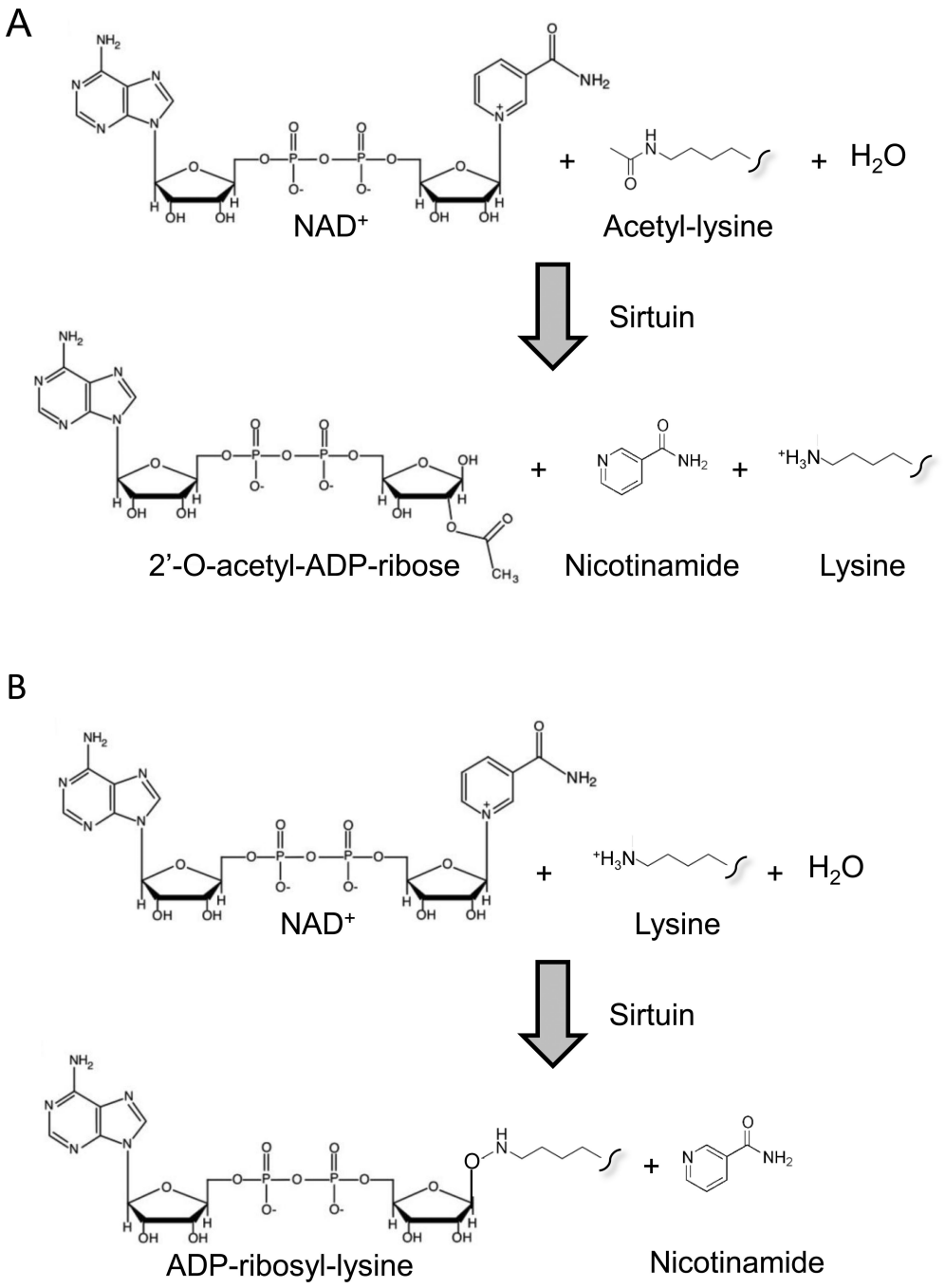

Figure 1.

Typical chemical reactions catalyzed by sirtuin enzymes. (A) Protein lysine deacetylation by sirtuins requires NAD^+ as a cofactor, releasing deaceylated protein, nicotinamide, and 2'-O-acetyl-ADP-ribose. (B) Certain sirtuins are ADP-ribosyltransferases, attaching the ADP-ribose moiety to the ε-amine of lysine, releasing nicotinamide.

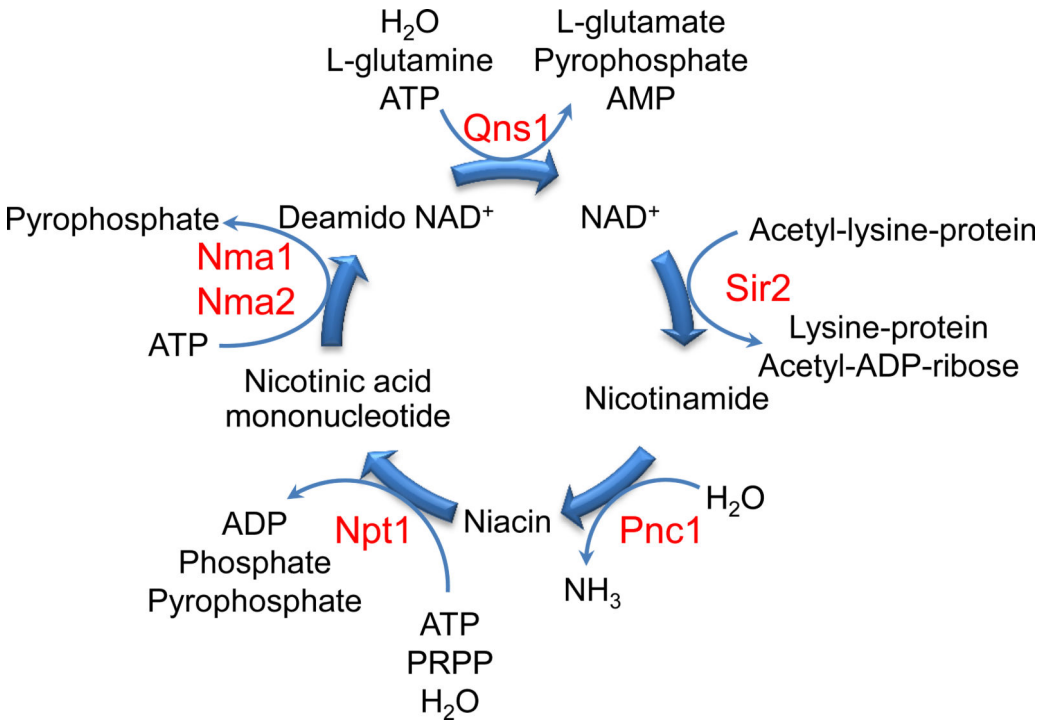

Figure 2.

NAD^+ salvage pathway recycles nicotinamide from the sirtuin catalyzed deacetylation reaction and regenerates NAD^+. Activation of this pathway may play an important role in modulating sirtuin activities by removing the sirtuin inhibitor nicotinamide and generating the cofactor NAD^+. Key yeast enzymes and metabolites in this pathway are shown.

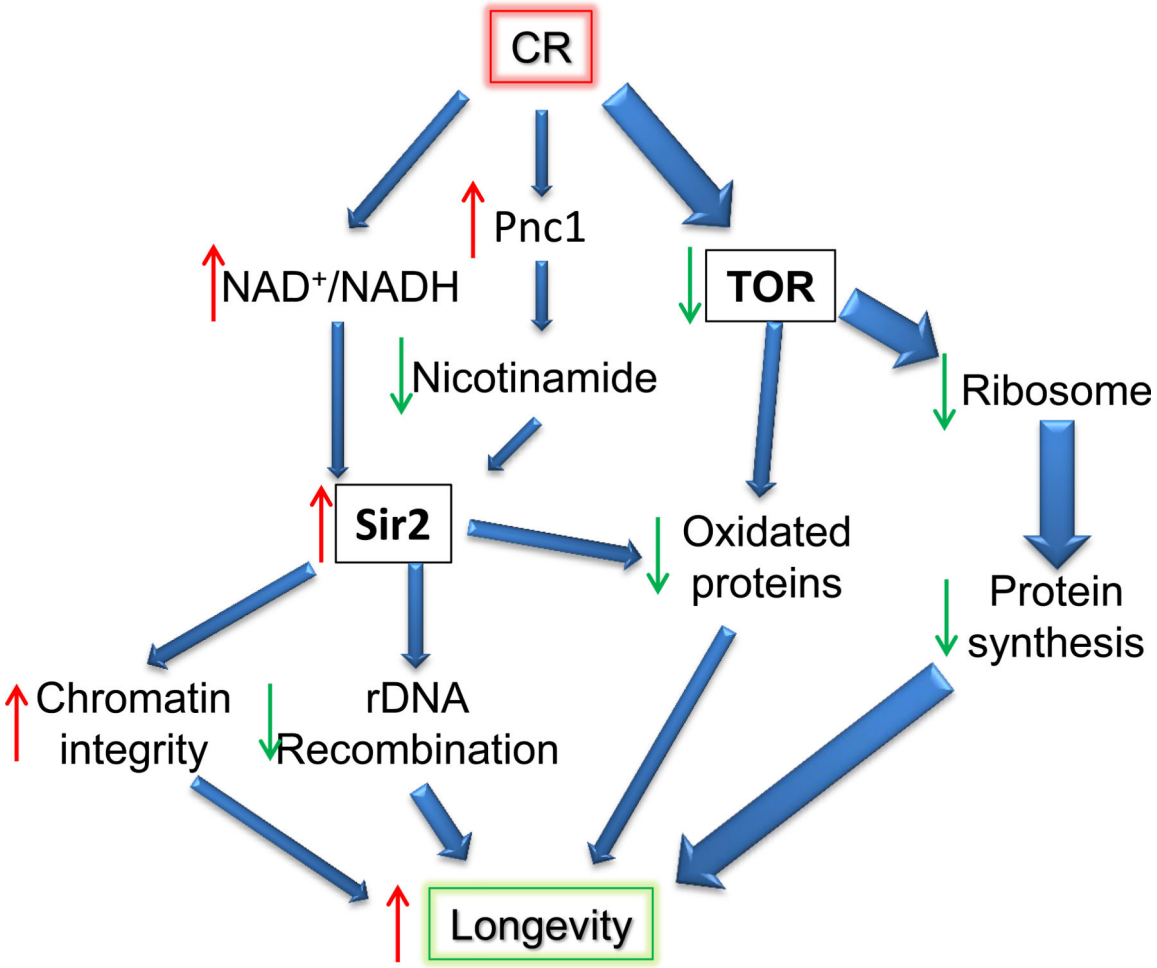

Figure 3.

A proposed mechanism of both sirtuins and TOR regulated pathways are modulated by the availability of nutrition states. The red “up” and green “down” arrows indicate increased and decreased activity or abundance, respectively. The thickness of the blue arrows indicates the relative impact to the downstream targets.

Table 1

Summary of sirtuin functions

	Localization	Activity	Target	Molecular and cellular function
Yeast				
Sir2	Nucleus, nucleolus	Deacetylation	H4K16, H3K56, H3K4	Gene silencing, heterochromatin
Hst1	Nucleus	Deacetylation	H3K4	Transcription repression
Hst2	Cytoplasm	Deacetylation	H4K16	Nucleolar and telomere silencing
Hst3	Nucleus	Deacetylation	H3K56	DNA replication and repair
Hst4	Nucleus	Deacetylation	H3K56	DNA replication and repair
Worm				
sir-2.1	Nucleus	Deacetylation	H3K9	Transcription silencing, heterochomatin
Fly				
dSir2	Nucleus, cytoplasm	Deacetylation	Histone, Dmp53	Transcription silencing, heterochomatin
Mammal				
SIRT1	Nucleus	Deacetylation	H1K26, H4K16, p53, PGClα, NF-κB, FOXO1, FOXO3, FOXO4, Notch, HIFlα, 14-3-3, PI3K, DNMT1, TORC1, HSF1, Ku70	Transcription silencing, mitochondria regulation, insulin signaling, tumorigenesis, apoptosis, cell proliferation and survival, tissue regeneration, differentiation, stress response
SIRT2	Cytoplasm	Deacetylation	H4K16, Tubulin, PAR-3, FOXO1, FOXO3, CDH1, CDC20, PGClα	Mitosis, nerve myelination and regeneration, brain aging, adipocyte differentiation, genome integrity, oxidative catabolism
SIRT3	Mitochondria	Deacetylation	LCAD, IDH2, GDH, ACS2, SOD2	Fatty acid oxidation, TCA cycle, oxidative phosphorylation, oxidative stress
SIRT4	Mitochondria	ADP-ribosylation	GDH	TCA cycle, fatty acid oxidation
SIRT5	Mitochondria	Deacetylation, demalonylation, desuccinylation	CPS1	Urea cycle
SIRT6	Nucleus	Deacetylation ADP-ribosylation	H3K9, H3K56, PARP1	Genome stability, telomere silencing
SIRT7	Nucleolus	Unknown	Unknown	rDNA transcription

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