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NAD+ and Sirtuins in Aging and Disease

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Abstract

Nicotinamide adenine dinucleotide (NAD^+) is a classical coenzyme mediating many redox reactions. NAD^+ also plays an important role in the regulation of NAD^+-consuming enzymes, including sirtuins, poly-ADP-ribose polymerases (PARPs), and CD38/157 ectoenzymes. NAD^+ biosynthesis, particularly mediated by nicotinamide phosphoribosyltransferase (NAMPT), and SIRT1 function together to regulate metabolism and circadian rhythm. NAD^+ levels decline during the aging process and may be an Achilles' heel, causing defects in nuclear and mitochondrial functions and resulting in many age-associated pathologies. Restoring NAD^+ by supplementing NAD^+ intermediates can dramatically ameliorate these age-associated functional defects, counteracting many diseases of aging, including neurodegenerative diseases. Thus, the combination of sirtuin activation and NAD^+ intermediate supplementation may be an effective anti-aging intervention, providing hope to aging societies worldwide.

Keywords

NAD^+; Sirtuins; Poly-ADP-ribose polymerases (PARPs); Nicotinamide phosphoribosyltransferase (NAMPT); Nicotinamide mononucleotide (NMN); Nicotinamide riboside (NR)

NAD^+ as an essential compound for many enzymatic processes

Nicotinamide adenine dinucleotide (NAD^+) was discovered more than a century ago by Sir Arthur Harden as a low molecular weight substance present in a boiled yeast extract, which could stimulate fermentation and alcohol production *in vitro* [1]. Subsequent studies over the

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next several decades determined that the structure of NAD^+ comprised two covalently joined mononucleotides (nicotinamide mononucleotide or NMN, and AMP), and identified the keystone function of NAD^+ and NADH as enzyme cofactors mediating hydrogen transfer in oxidative or reductive metabolic reactions [1].

For an extended period, NAD^+ thus appeared in biochemistry textbooks with the sole function of a cofactor of enzymes serving metabolic pathways in cells. More recently, NAD^+ has been associated with biochemical reactions other than hydrogen transfer, serving as a cosubstrate for bacterial DNA ligase [2], poly-ADP-ribose polymerase or PARP [3], CD38/157 ectoenzymes [4], and class III NAD^+-dependent deacylases or sirtuins [5]. In all of these newer examples, NAD^+ is cleaved at the glycosidic bond between nicotinamide and ADP ribose (Figure 1, described in detail, below). For the ligase, ADP ribose is transferred to the 5′ hydroxyl of DNA to be ligated. For PARP, ADP ribose is serially transferred to arginine side chains in itself, histones, and other proteins at sites of DNA damage. For CD38/157, NAD^+ is provided through the connexin 43 hemichannels and hydrolyzed extracellularly. These enzymes also generate cyclic ADP-ribose, a strong Ca2+ inducer. Lastly, for sirtuins, NAD^+ cleavage catalyzes the removal of acetyl or acyl groups from lysines of sirtuin substrate proteins accompanied by their transfer to ADP ribose.

Much excitement arose from the idea that sirtuins regulate health and life span in many different organisms in accord with diet. In particular, it was shown that NAD^+ and NADH could vary with the availability of dietary energy and nutrients. For example, an increase in NAD^+ (or decrease in NADH) was proposed to mediate the extension of life and health span by dietary restriction (DR) [6]. This study challenged the dogma arising from earlier studies, which found that NAD^+ was present in excess to NADH in cells and did not vary much with diet [7]. Reciprocally, many recent studies have provided evidence that defects in maintaining NAD^+ levels and the accompanying decline in activity of sirtuins may help drive normal aging [8, 9]. These latter studies are additionally exciting because they also demonstrate that NAD^+ deficiency and associated pathologies may be normalized by supplementation with NAD^+ precursors and intermediates. This review expands on this new framework, considering aging and diseases, and discusses the emergence of approaches to counter effects of aging by small molecules that can rescue defects in NAD^+ and sirtuin activity.

NAD^+ plays a key role in regulating metabolism and circadian rhythm

The canonical role of NAD^+, mentioned above, is to facilitate hydrogen transfer in key metabolic pathways (Figure 1a). For example, NAD^+ is converted to NADH in the glyceraldehyde-3-phosphate dehydrogenase step of glycolysis, a pathway in which glucose is converted to pyruvate. Conversion of NAD^+ to NADH is also important in mitochondrial metabolism. In that compartment, NAD^+ is converted to NADH in four steps of the mitochondrial TCA cycle, in which acetyl-CoA is oxidized to carbon dioxide. NAD^+ is also converted to NADH during the oxidation of fatty acids and amino acids in mitochondria. In these mitochondrial pathways, the NADH generated is an electron donor for oxidative phosphorylation and ATP synthesis.

In addition to these canonical uses of NAD^+ and NADH, PARPs transfer ADP-ribose from NAD^+ to itself, histones, and other proteins at sites of DNA damage to facilitate repair and maintenance of genomic integrity (Figure 1b). Damaged DNA recruits PARP and activates its poly-ADP-ribosylation activity *in situ*. Thus, acute DNA damage, for example by ionizing radiation, can trigger a sudden depletion of NAD^+ due to PARP activation. PARP inhibitors are in clinical trials as anti-cancer agents [10], because they can sensitize tumor cells to apoptotic killing by genotoxic agents through the prevention of DNA repair.

Sirtuins are NAD^+-dependent deacylases, which play key roles in responding to nutritional and environmental perturbations, such as fasting, DR, DNA damage, and oxidative stress (Figure 1c). In general, their activation triggers nuclear transcriptional programs that enhance metabolic efficiency and also upregulate mitochondrial oxidative metabolism and the accompanying resistance to oxidative stress [11]. Sirtuins foster this resistance by increasing anti-oxidant pathways (e.g. SOD2 and IDE2 in mitochondria) and by facilitating DNA damage repair through deacetylation or ADP-ribosylation of repair proteins [12]. Accordingly, many studies have shown that sirtuins promote longevity in yeast, worms, flies, and mice, and can mitigate many diseases of aging in murine models, such as type 2 diabetes, cancer, cardiovascular diseases, neurodegenerative diseases, and pro-inflammatory diseases [11, 13, 14]. Although a challenge was raised to the proposed conserved role of sirtuins in aging/longevity control [15] (Box 1), many recent studies have upheld the original claims [16–23].

Box 1

The role of sirtuins in aging and longevity control

Early studies have demonstrated that Sir2 and its orthologs play an important role in aging/longevity control in diverse model organisms including yeast, worms, and flies (24–26). In those organisms, it has also been shown that Sir2 and its orthologs mediate caloric restriction-induced lifespan extension in certain genetic backgrounds (25, 27–30). Although many studies have reported that SIRT1, the mammalian ortholog of Sir2, mediates anti-aging effects of caloric restriction in mice (13), mice overexpressing SIRT1 in the whole body failed to show lifespan extension (31). Furthermore, previous results showing lifespan extension by Sir2 orthologs in worms and flies were called into question (15), bringing considerable debate around the importance of sirtuins in aging/longevity control to the field of aging research. However, more recently, an increasing number of studies have reconfirmed the original claims (16–23). In mammals, it has been reported that whole-body Sirt6 transgenic mice show lifespan extension, in males (17). Most recently, it has been demonstrated that increasing SIRT1 specifically in the brain, particularly in the dorsomedial and lateral hypothalamic nuclei, delays aging and extends lifespan in both male and female mice (20). These new studies have thus put the controversy to rest, and provide a firmer foundation for the importance of sirtuins as an evolutionarily conserved aging/longevity regulator.

Among the many ways sirtuins influence metabolism is by regulating the circadian clock machinery. SIRT1, the most studied member of mammalian sirtuins, deacetylates central clock components in the liver [32, 33], and also amplifies the expression of the circadian transcription factors BMAL and CLOCK in the suprachiasmatic nucleus (SCN) of the hypothalamus via deacetylation of PGC-1α [34]. In the latter case, a loss of SIRT1 function occurs with aging, which results in damped levels of the clock components and deterioration of central circadian control. Defects in central circadian control have been associated with disease and premature aging, underscoring the metabolic importance of circadian function [35].

Reciprocally, NAD^+ synthesis is regulated by the circadian machinery to provide a critical link from the clock oscillator to metabolic pathways [36]. In this regard, one must remember that NAD^+ synthesis encompasses both *de novo* and salvage pathways, with some differences between lower organisms and mammals (Figure 2). Importantly, one of the key target genes of BMAL and CLOCK is the rate-limiting enzyme for NAD^+ biosynthesis from nicotinamide, nicotinamide phosphoribosyltransferase or NAMPT [37, 38]. NAD^+ is synthesized in a circadian oscillatory fashion systemically, leading to a circadian schedule of sirtuin activation and mitochondrial metabolism, such as oxidation of fatty acids [39]. Any decline in central and peripheral circadian function with aging would thus degrade the temporal order of metabolism, which may contribute to a deterioration in health.

Finally, NAD^+ is used in cells to generate other important bioactive derivatives, such as cyclic ADP ribose (cADPR) and 1-methylnicotinamide (Figures 1d and 2). cADPR is generated (and can be hydrolyzed) by CD38 and its relative CD157, and mutations in CD38 not only lower production of cADPR but also substantially raise NAD^+ levels in mice [40, 41]. cADPR can play an important role in signaling by stimulating intracellular calcium release, and the range of its biological functions are just beginning to be uncovered [42]. 1-methylnicotinamide is made by nicotinamide-N-methyltransferase from the NAD^+ cleavage product nicotinamide (Figure 2). A recent study has shown that 1-methylnicotinamide plays an important role in the extension of worm life span by the sirtuin SIR-2.1, the ortholog of mammalian SIRT1 [21].

NAD^+ declines with aging and can be restored by supplementation with NAD^+ precursors

Several studies have reported that the activity of sirtuins decays with aging [34, 43, 44]. The mammalian Sir2 ortholog SIRT1 can be regulated by many mechanisms, including transcriptionally, and post-translationally by changes in stability, phosphorylation, SIRT1-binding proteins, and by changes in NAD^+ levels [14]. Of these mechanisms regulating SIRT1, a systemic decline in NAD^+ has emerged as a likely explanation for why aging affects sirtuins. The decline in NAD^+ was first noticed in transgenic mice overexpressing SIRT1 in pancreatic β cells (BESTO mice) [44]. BESTO mice showed enhanced glucose-stimulated insulin secretion when they were young, but lost this phenotype when they became old. Importantly, administration of a key NAD^+ intermediate, nicotinamide mononucleotide (NMN), restored the metabolic phenotype in old BESTO mice and enhanced insulin secretion in old wild-type control mice. Note NMN can be converted into NAD^+ by NMN adenylyltransferases (NMNATs) in one step (Figure 2). This finding suggests that a decrease in NAD^+ with aging was responsible for the loss of the phenotype in pancreatic β cells of BESTO mice. Consistent with this surmise, NAD^+ levels have been shown to decline approximately 2-fold in old worms and in multiple tissues, including liver and skeletal muscle, in aged mice [18, 43, 45].

Another supplementation study with NMN has been shown to restore NAD^+ levels and prevent diet- and age-induced type 2 diabetes in wild-type mice [45]. In a recent study, NMN was reported to dramatically reverse the effects of aging at the cellular and organismal levels [43]. Another NAD^+ intermediate, nicotinamide riboside (NR), can also be converted to NAD^+, after conversion to NMN via NR kinase (Nrk) [46, 47] (Figure 2). Like NMN, NR boosts NAD^+ levels in worms and mice and can counter effects of aging [18, 48]. NR supplementation also increases mitochondrial NAD^+ levels and stimulates SIRT3-mediated deacetylation of mitochondrial proteins [48].

Importantly, NAD^+ intermediate supplementation appears to restore NAD^+ levels in both nuclear and mitochondrial compartments of cells. In one study, aging was shown to trigger SIRT1 inactivation, which was reversed by NMN, demonstrating supplementation of an NAD^+ deficiency in the nuclear/cytosolic pool [43]. In another study, a mitochondrial deficiency in complex I of the electron transport chain led to depletion of mitochondrial NAD^+ due to accumulation of NADH, inactivation of the mitochondrial SIRT3, and severe cardiac damage [49]. These effects could also be corrected by supplementation with NMN [49]. Thus, the benefits of NAD^+ intermediate supplementation appear to be due to reactivation of sirtuins. Alternatively, reactivation of other NAD^+-dependent enzymes may be critical in improving health by this supplementation.

Possible mechanisms for how NAD^+ levels decline in aging

Why do NAD^+ levels decline with aging? One possibility is that one or more of the NAD^+ biosynthetic pathways decline. Indeed, there is some evidence that levels of NAMPT decline during aging [45], whereas exercise training has the opposite effect, at least in skeletal muscle [50]. Moreover, as discussed above, NAMPT is a major output of the circadian transcription factors BMAL and CLOCK. If the activity of the circadian machinery systemically declined with aging, as appears to be the case in the SCN [34], a deficit in NAMPT and NAD^+ would result (Figure 3). Under such conditions, the use of NAD^+ intermediates, such as NMN and NR, rather than earlier NAD^+ precursors like nicotinamide, would be critical to enhance NAD^+ biosynthesis efficiently in aged individuals.

Interestingly, it has been shown that tumor necrosis factor-α (TNF-α), one of major inflammatory cytokines, and oxidative stress significantly reduce NAMPT and NAD^+ levels in primary hepatocytes [45]. TNF-α also suppresses CLOCK/BMAL-mediated clock gene transcription in the liver and SCN of TNF-α-treated mice [51]. Since both inflammatory cytokines and oxidative stress contribute to the development of chronic inflammation during aging [52], chronic inflammation could be a reason by which both NAMPT-mediated NAD^+ biosynthesis and CLOCK/BMAL-mediated circadian machinery are compromised during aging (Figure 3). If found true, strategies to suppress chronic inflammation and sustain NAD^+ biosynthesis and circadian function with aging might be effective in maintaining sirtuin activity and possibly robust health [9].

A second mechanism of NAD^+ decline was suggested by analysis of PARP1 knockout mice [53]. There was a systemic elevation in NAD^+ levels, SIRT1 activity, and metabolic benefits in these mice. Moreover, chemical inhibitors of PARP1 exerted similar effects. Parallel findings were also reported for mice with a knock out in another NAD^+-consuming enzyme, CD38, as shown previously [54, 55]. These studies show clearly that PARP, CD38 and the nuclear sirtuins all compete for the same pool of NAD^+, and inhibition of PARP or CD38 has the potential of activating sirtuins.

Nevertheless, how does this relate to the decline in NAD^+ with aging? A recent study showed that PARP was chronically activated in aging worms and mice (liver or skeletal muscle), leading to an increase in poly-ADP-ribosylation of cellular proteins [18]. Moreover, PARP activation closely corresponds to reduced NAD^+ levels and increased acetylation of a canonical SIRT1 substrate, PGC-1α. This follows findings that knockout mutations in PARP1 increase NAD^+ levels and SIRT1 activity in mice [53]. A possible explanation for these findings is that aging is associated with an increase in chronic nuclear DNA damage, which leads to NAD^+ depletion by PARP (Figure 4, left). The fact that loss of SIRT1 or SIRT6 activity exacerbates DNA damage [12] may create an autocatalytic downward spiral in the nucleus with NAD^+ depletion as the nexus.

Mitochondria as a common target of aging-induced NAD^+ decline

It is now clear that aging-induced inactivation of SIRT1 has a direct and deleterious effect on mitochondria, as first suggested by the important associations between SIRT1 and PGC-1α [56] and SIRT1 and TFAM [43]. A reduction in SIRT1 activity downregulates mitochondrial biogenesis, oxidative metabolism, and associated anti-oxidant defense pathways, leading to damage to complex I of the electron transport chain and a decline in mitochondrial function (Figure 4, right). A similar effect could result from the failure of SIRT1 to deacetylate another of its substrates, FOXO, which would lead to a reduction in mitochondrial anti-oxidant defenses in worms [57] and mammals [58].

Strikingly, other mechanisms have also been recently unveiled, which connect sirtuins to mitochondrial health. Inducing the activity of the worm SIR-2.1 or mammalian SIRT1 triggers the mitochondrial unfolded protein response (UPR^{mt}) pathway, but not other protein quality control pathways, such as those affecting the endoplasmic reticulum [18]. Indeed, genetic inactivation of the UPR^{mt} pathway prevents the longevity induced by SIR-2.1 overexpression or by NR supplementation in worms. Recently, it has also been reported that SIRT3 regulates the UPR^{mt} and mitophagy [59]. Thus, clearance of damaged mitochondria may also be impaired by NAD^+ deficiency.

Finally, a defect in expression of mitochondrial-encoded proteins in skeletal muscle of 24-month old mice (only at older ages was a reduction in nuclear encoded mitochondrial proteins also observed) was shown to lead to metabolic decline [43]. Depressed mitochondrial gene expression and metabolic decline were due to a defect in SIRT1 activity and were reversed by supplementation with NMN. Thus, NAD^+ deficiency again appears to be the primary trigger, in this case reducing mitochondrial gene expression. Surprisingly, this defect arising from SIRT1 inactivation was not related to PGC-1α or the UPR^{mt}. Rather, SIRT1 deficiency prevented its known downregulation of HIF-1α, leading to an inappropriately high level of HIF-1α. This pseudohypoxic state led to sequestration of cMYC by HIF-1α. Thus, cMYC could no longer activate the promoter of the gene for the mitochondrial transcription factor TFAM. Importantly, knocking out SIRT1 in skeletal muscle of young mice recapitulated many of these effects of normal aging.

The connection between low NAD^+ pools in the nucleus and the various mitochondrial quality control mechanisms is noteworthy, because mitochondrial dysfunction is a hallmark of aging [60]. Moreover, these findings provide a link by which a nuclear NAD^+ defect, for example due to PARP activation, may also affect the mitochondrial pool of NAD^+. A decline in SIRT1 activity thus leads to mitochondrial dysfunction and compromises electron transport. A buildup of the substrate of electron transport, NADH, at the expense of mitochondrial NAD^+ is a necessary consequence. To further the problem, a mitochondrial NAD^+ deficiency will inactivate mitochondrial sirtuins, again leading to an autocatalytic downward spiral in this compartment. The fact that NAD^+ intermediate supplementation can affect both the nuclear and mitochondrial NAD^+ pools is critical to the efficacy of these compounds in health maintenance.

Prospects for treating neurodegenerative diseases?

Transgenic mice overexpressing SIRT1 throughout the body have been shown to counteract detrimental effects of energy-dense diet and aging and also mimic some physiological phenotypes induced by DR [11]. Furthermore, SIRT1 transgenic mice overexpressing this protein in the brain are protected in mouse models of Alzheimer's disease [61, 62], Parkinson's disease [63] and Huntington's disease [64, 65]. In another mouse model, Wallerian degeneration slow (WldS) mice owe their heightened protection against peripheral nerve degeneration upon injury to triplication of the NMNAT1 gene [66–68]. Thus, SIRT1 and NAD^+ may be broadly neuro-protective. However, in most of the above studies, the degree of protection by SIRT1 overexpression or resveratrol is at best partial. It seems likely that NAD^+ depletion may occur in at least a subset of the neurodegenerative diseases. This hypothesis follows from the observation that these diseases have been associated with an increase in chronic nuclear DNA damage [69, 70]. If NAD^+ is depleted, then protection by SIRT1 activation could be limited and could decline altogether as the disease progressed and NAD^+ levels fall below the Km for SIRT1.

It is of interest that transgenic mice modeled for Alzheimer's disease are partially protected against memory loss by NR supplementation [71]. NR supplementation was associated with an increase in PGC-1α and a decrease in the β-secretase, which generates the toxic amyloid-β peptide. Although SIRT1 was not monitored, it seems a likely immediate target for the effect of NR. Therefore, it is of interest to determine whether NAD^+ declines in one, some, or all of the neurodegenerative diseases and whether supplementation of NAD^+ intermediates, such as NMN and NR, for the restoration of NAD^+ will be broadly beneficial. If so, it will be essential to revisit the effects of SIRT1 activation, either by transgenes or by compounds, in combination with NAD^+ intermediate supplementation. There is currently no effective treatment for any of these neurodegenerative diseases, which continue to arise in an increasingly long-lived population. A broad therapy to treat a number of these diseases would be transformative, and undoubtedly, no stone should be left unturned to find one. The combination of sirtuin activation and NAD^+ intermediate supplementation to restore NAD^+ may be an intriguing way to start down one such path.

Concluding remarks

Recent studies have indicated that NAD^+ decline may drive aging through decreased sirtuin activities in the nucleus and mitochondria. NAD^+ decline might be caused by the defect in NAMPT-mediated NAD^+ biosynthesis and the PARP-mediated depletion of NAD^+, both of which appear to occur during the aging process and perhaps in age-associated diseases, including neurodegenerative diseases. Supplementation of key NAD^+ intermediates, such as NMN and NR, can ameliorate a variety of age-associated pathophysiologies generated by NAD^+ decline. Further investigations will be necessary to clarify outstanding questions that remain in the field (Outstanding questions box).

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Outstanding questions

- Does declining NAD^+ contribute to aging only because it inactivates sirtuins?
- Will NAD^+ intermediate supplementation treat neurodegenerative diseases, as well as other age-associated diseases in rodent models?
- Will NAD^+ supplementation synergize with SIRT1 activating compounds?
- Will NAD^+ intermediate supplementation be efficacious in humans?

Highlights

- NAD^+plays a key role in regulating metabolism and circadian rhythm through sirtuins.
- NAD^+becomes limiting during aging, affecting sirtuin's activities.
- NAD^+likely declines due toan NAD $^+$biosynthesis defect and increased depletion.
- Supplementing key NAD^+ intermediates can restore NAD^+ levels and ameliorate age-associated pathophysiologies.

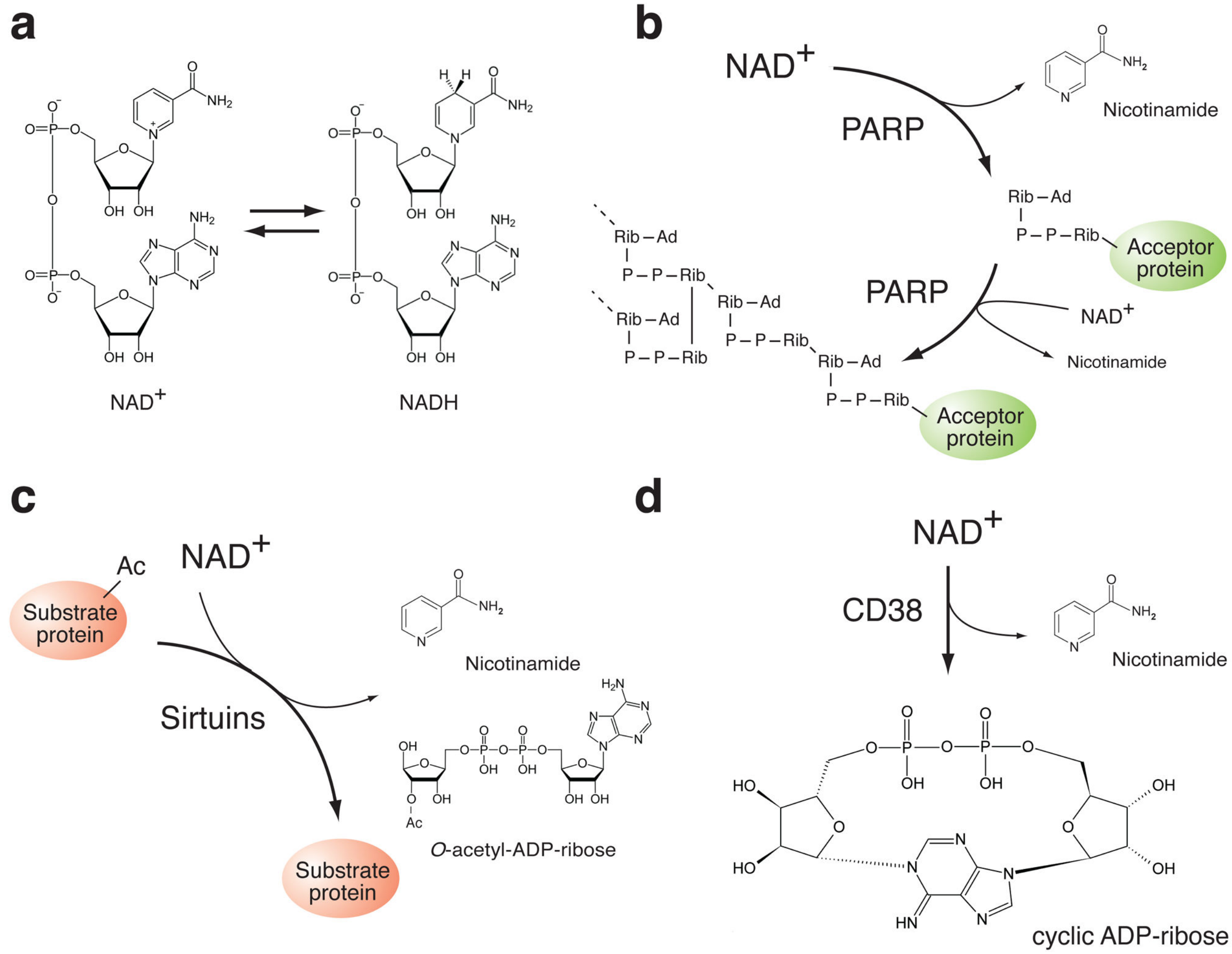

Figure 1.

Various uses of NAD^+ for canonical redox and NAD^+-consuming enzymatic reactions. Whereas NAD^+ is converted to NADH by many metabolic enzymes (a), it is also used as a cosubstrate for NAD^+-consuming enzymes, such as poly-ADP-ribose polymerases (PARPs) (b), sirtuins (c), and CD38/157 ectoenzymes (d).

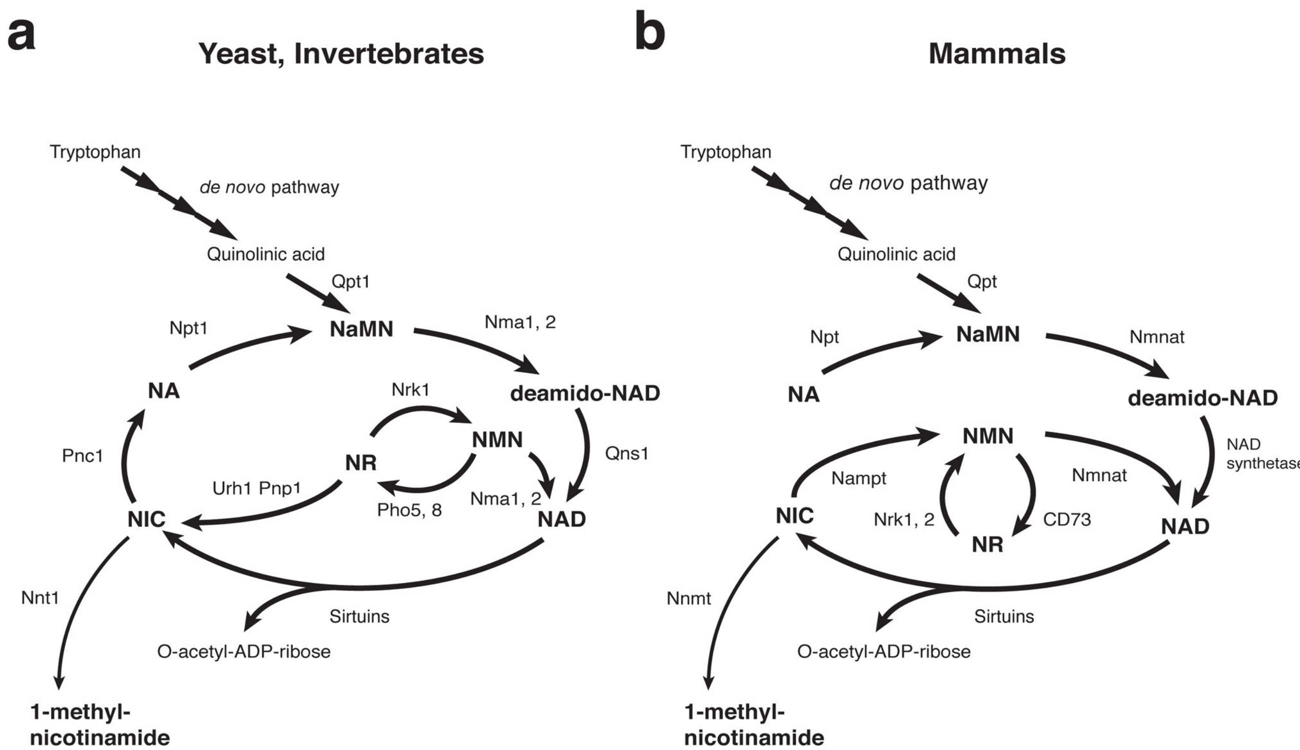

Figure 2.

NAD$^+$ biosynthetic pathways in various organisms. (a) The *de novo* pathway from tryptophan and the salvage pathway through nicotinamide (NIC) and nicotinic acid (NA) in the budding yeast *Saccharomyces cerevisiae*. These pathways are also conserved in invertebrates. Pnc1, nicotinamidase; Npt1, nicotinic acid phosphoribosyltransferase; Nma1, 2, nicotinic acid mononucleotide adenylyltransferase 1, 2; Qns1, NAD synthetase; Qpt1, quinolinic acid phosphoribosyltransfease; Nrk1, nicotinamide ribose kinase 1; Pho5, 8, phosphatase 5, 8; Urh1, Pnp1, nucleosidases; Nnt1, nicotinamide-N-methyltransferase. (b) NAD$^+$ biosynthetic pathways in mammals. In mammals, NAD$^+$ can be synthesized from tryptophan, nicotinic acid (NA) and nicotinamide (NIC) (two forms of vitamin B3), and nicotinamide riboside (NR). NIC is a predominant NAD$^+$ precursor in mammals. The *de novo* pathway and the NAD$^+$ biosynthetic pathway from nicotinic acid are evolutionarily conserved, whereas the NAD$^+$ biosynthetic pathway from nicotinamide is mediated by nicotinamide phosphoribosyltransferase (Nampt). While multiple enzymes break NAD$^+$ into nicotinamide and ADP-ribose, only sirtuins are shown in this figure. The resultant NIC is also converted to 1-methylnicotinamide by nicotinamide-N-methyltransferase (Nnmt). Mammals have two NR kinases (Nrk1 and 2) and ecto-5′-nucleotidase CD73 to produce NMN and NR, respectively. NaMN, nicotinic acid mononucleotide; NMN, nicotinamide mononucleotide.

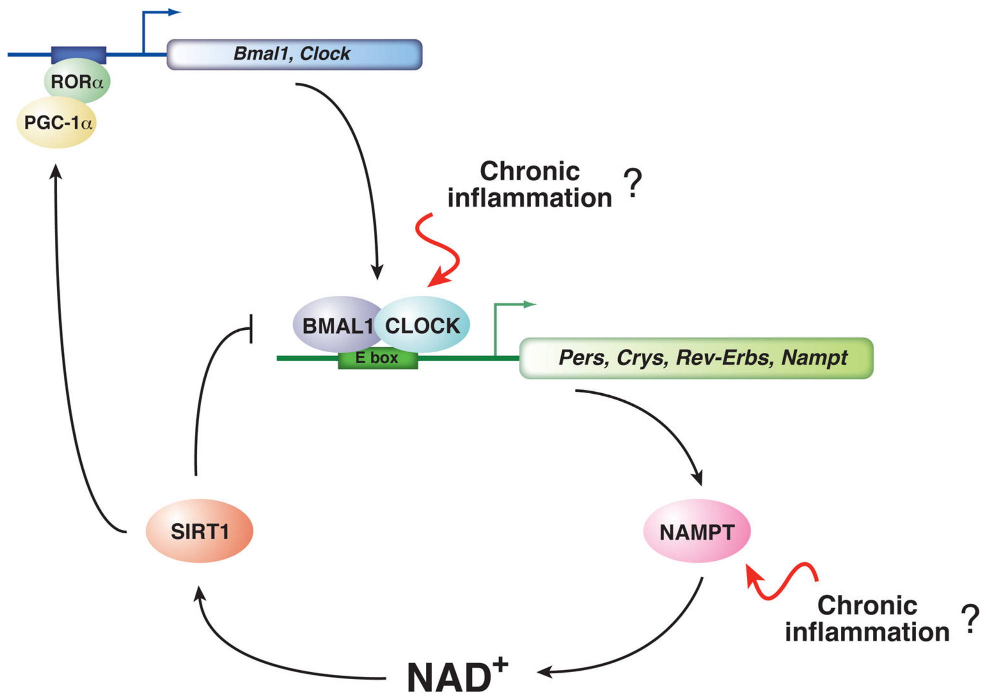

Figure 3.

Synthesis of NAD$^+$ is regulated by the circadian clock and declines with age. The oscillating clock consists of the heterodimeric complex of core circadian transcription factors BMAL1 and CLOCK. The BMAL1/CLOCK complex controls the *Nampt* gene encoding the key NAD$^+$ biosynthetic enzyme nicotinamide phosphoribosyltransferase (NAMPT), rendering NAD$^+$ production and SIRT1 activity circadian in peripheral tissues. SIRT1 negatively regulates the transcriptional activity of the BMAL1/CLOCK complex, completing a novel circadian-regulatory feedback loop. In the suprachiasmatic nucleus (SCN), SIRT1 also regulates *Bmal1* and *Clock* expression levels via the complex with PGC-1α and RORα. Chronic inflammation, particularly induced by inflammatory cytokines such as TNF-α, might affect NAMPT-mediated NAD$^+$ biosynthesis and BMAL1/CLOCK-mediated circadian transcription in peripheral tissues and the SCN, causing a decline in the amplitude of the circadian clock with age.

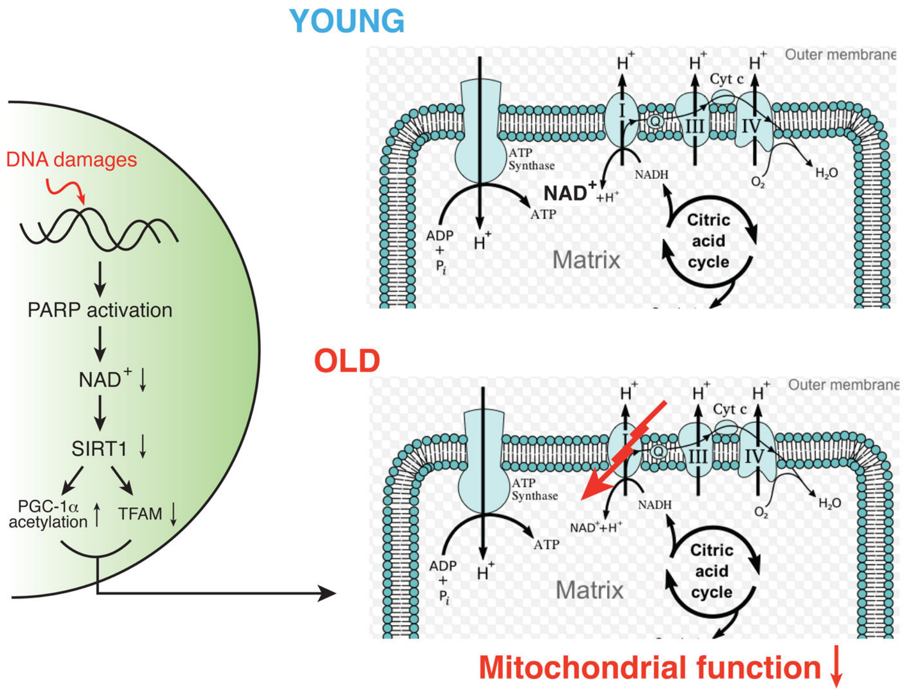

Figure 4.

Electron transport via NADH generates NAD^+ in mitochondria and may decline with age. In young mitochondria, NADH, made by the citric acid cycle, readily donates its electrons to complex I of the electron transport chain (ETC) and thereby generates NAD^+. During the aging process, DNA damage accumulates in the nucleus, causing PARP activation and NAD^+ reduction. Consequently, SIRT1 activity is reduced, resulting in increased PGC-1α acetylation and decreased TFAM levels. These nuclear events might reduce mitochondrial function in old mitochondria by affecting mitochondrial complex I and other mitochondrial components, or blocking the entry of electrons from NADH into the ETC, thereby creating an NAD deficiency.