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Nicotinamide Adenine Dinucleotide Metabolism and Neurodegeneration

Mariana Pehar, Benjamin A. Harlan, Kelby M. Killoy, and Marcelo R. Vargas

Abstract

Significance: Nicotinamide adenine dinucleotide (NAD^+) participates in redox reactions and NAD^+-dependent signaling processes, which involve the cleavage of NAD^+ coupled to posttranslational modifications of proteins or the production of second messengers. Either as a primary cause or as a secondary component of the pathogenic process, mitochondrial dysfunction and oxidative stress are prominent features of several neurodegenerative diseases. Activation of NAD^+-dependent signaling pathways has a major effect in the capacity of the cell to modulate mitochondrial function and counteract the deleterious effects of increased oxidative stress.
Recent Advances: Progress in the understanding of the biological functions and compartmentalization of NAD^+-synthesizing and NAD^+-consuming enzymes have led to the emergence of NAD^+ metabolism as a major therapeutic target for age-related diseases.
Critical Issues: Three distinct families of enzymes consume NAD^+ as substrate: poly(ADP-ribose) polymerases (PARPs), ADP-ribosyl cyclases (CD38/CD157) and sirtuins. Two main strategies to increase NAD^+ availability have arisen. These strategies are based on the utilization of NAD^+ intermediates/precursors or the inhibition of the NAD^+-consuming enzymes, PARPs and CD38. An increase in endogenous sirtuin activity seems to mediate the protective effect that enhancing NAD^+ availability confers in several models of neurodegeneration and age-related diseases.
Future Directions: A growing body of evidence suggests the beneficial role of enhancing NAD^+ availability in models of neurodegeneration. The challenge ahead is to establish the value and safety of the long-term use of these strategies for the treatment of neurodegenerative diseases. *Antioxid. Redox Signal*. 28, 1652–1668.

Keywords: NAD, mitochondria, oxidative stress, neurodegeneration, CD38, PARP

Introduction

Nicotinamide adenine dinucleotide (NAD^+) has long been recognized as an essential redox molecule in metabolism. However, its role as cosubstrate for a series of enzymes that regulate fundamental biological processes has recently renewed the interest on this dinucleotide. In redox reactions, a hydride equivalent is reversibly transferred at the nicotinamide moiety causing a switch between oxidized (NAD^+) and reduced (NADH) forms of the nucleotide. While this property of NAD^+ is critical for redox reactions, it is not accompanied by net consumption of the dinucleotide. In contrast, NAD^+-dependent signaling involves the cleavage of NAD^+ (degradation) coupled to posttranslational modifications of proteins or the production of second messengers (5, 14, 78).

Activation of NAD^+-dependent signaling pathways has a major effect in the capacity of the cell to modulate mitochondrial function and counteract the deleterious effects of increased oxidative stress. Either as a primary cause or as a secondary component of the pathogenic process, mitochondrial dysfunction and oxidative stress are prominent features of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) (25, 45, 46, 121, 146, 155). As we learn more about the biological role of NAD^+-synthesizing and NAD^+-consuming enzymes and their compartmentalization, NAD^+ metabolism has emerged as a key therapeutic target for neurodegenerative diseases.

Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, South Carolina.

NAD+-Dependent Signaling

NAD^+ is a dinucleotide generated by the covalent link between nicotinamide mononucleotide (NMN) and adenosine monophosphate. In redox reactions, the addition of two electrons and a proton to the nicotinamide moiety of NAD^+ generates NADH (Fig. 1). This electron carrier function is critical for catabolic reactions and energy production in the cell but does not cause any net loss of NAD^+ because it is reversible. On the contrary, NAD^+-dependent signaling processes lead to its degradation. Three distinct families of enzymes consume NAD^+ as substrate: poly(ADP-ribose) polymerases (PARPs), ADP-ribosyl cyclases, and sirtuins (Fig. 2).

Poly(ADP-ribose) polymerases

In humans, the PARP family of proteins consists of 17 proteins widely distributed in all tissues. PARPs hydrolyze NAD^+ and transfer the ADP-ribose moiety to a receptor amino acid. PARPs transfer multiple ADP-ribose moieties leading to the formation of long, linear, or branched poly(ADP-ribose) polymers (Fig. 2). This family also includes members with mono-ADP-ribosylation activity, catalyzing the transfer of a single ADP-ribose moiety. PARPs regulate DNA damage repair, tumorigenesis, cell differentiation, and metabolism (14, 15, 36). PARP1 and PARP2 account for the majority of the basal and stimulated PARP activity in the cell (36). PARP1 activation is an integral part of the cellular response to oxidative stress-induced DNA damage.

Excessive activation of PARPs rapidly impairs mitochondrial function and is linked to cell death in a variety of situations. Multiple signaling pathways have been described to mediate the deleterious effect of PARP1 overactivation. In principle, PARP1 overactivation could lead to a catastrophic decrease in cytosolic NAD^+, leading to direct glycolytic inhibition and cell death (6, 156). Energy depletion following PARP1 overactivation could also be mediated by poly(ADP-ribose)-dependent inhibition of the enzyme hexokinase (9). In addition, cell death induced by PARP1 overactivation can be mediated by poly(ADP-ribose)-dependent release of apoptosis inducing factor from the mitochondria, part of a specific caspase-independent cell death program known as parthanatos (177, 189, 190).

In addition, it has been shown that the expression of a mitochondria-targeted PARP1 decreases total cellular NAD^+ and mitochondrial function, even in the presence of a compensatory increase in glycolytic activity (116). This result suggests that increased mitochondrial PARP activity is sufficient to directly impair mitochondrial function. Although PARP1 is predominantly localized to the nucleus, and despite the fact that PARP activity may or may not exist in the mitochondrial matrix, PARP activation remains a major component in oxidant-induced mitochondrial dysfunction (14, 29).

ADP-ribosyl cyclases

These unique enzymes catalyze the cyclization of NAD^+ to cyclic ADP ribose (cADPR) (Fig. 2). The two members of this family, CD38 and CD157, have receptor functions in immune and myeloid cells (133), but are also multifunctional enzymes that catalyze the production of several second messengers (such as cADPR, adenosine dinucleotide phosphoribose, and nicotinic acid adenine dinucleotide phosphate) (100). These products then act as potent intracellular calcium-mobilizing agents to control chemotaxis of dendritic cells and activation of microglia (108, 124).

Despite this original role described in cells of the immune system, CD38 is ubiquitously expressed in mammalian tissues and its major enzymatic activity is the hydrolysis of NAD^+. In fact, it has been estimated that CD38 will generate one molecule of cADPR for every 100 molecules of NAD^+ hydrolyzed (52). CD38 is highly expressed in neurons and astrocytes (125, 184).

Originally described as an ectoenzyme, CD38 is also present in the endoplasmic reticulum and in the nuclear and mitochondrial membranes (5), suggesting that CD38 could be an important regulator of intracellular NAD^+ pools, and therefore metabolic pathways, in both cell types. In the mouse central nervous system (CNS), the neuronal distribution of CD38 coincides with that of ryanodine receptors, supporting the involvement of CD38 in a cADPR-mediated

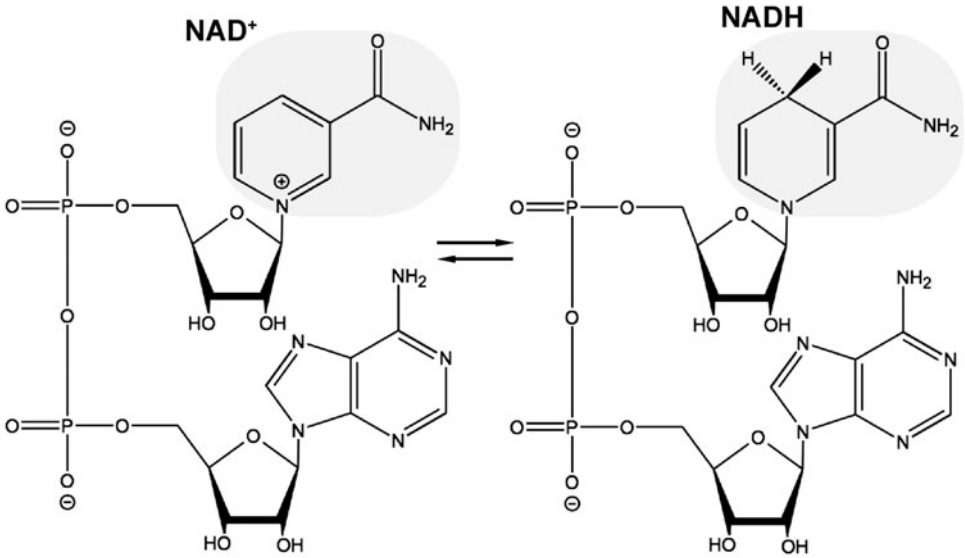

FIG. 1. NAD^+ as an electron carrier. In redox reactions, a hydride equivalent is reversibly transferred at the nicotinamide moiety causing a switch between oxidized (NAD^+) and reduced (NADH) forms of the dinucleotide.

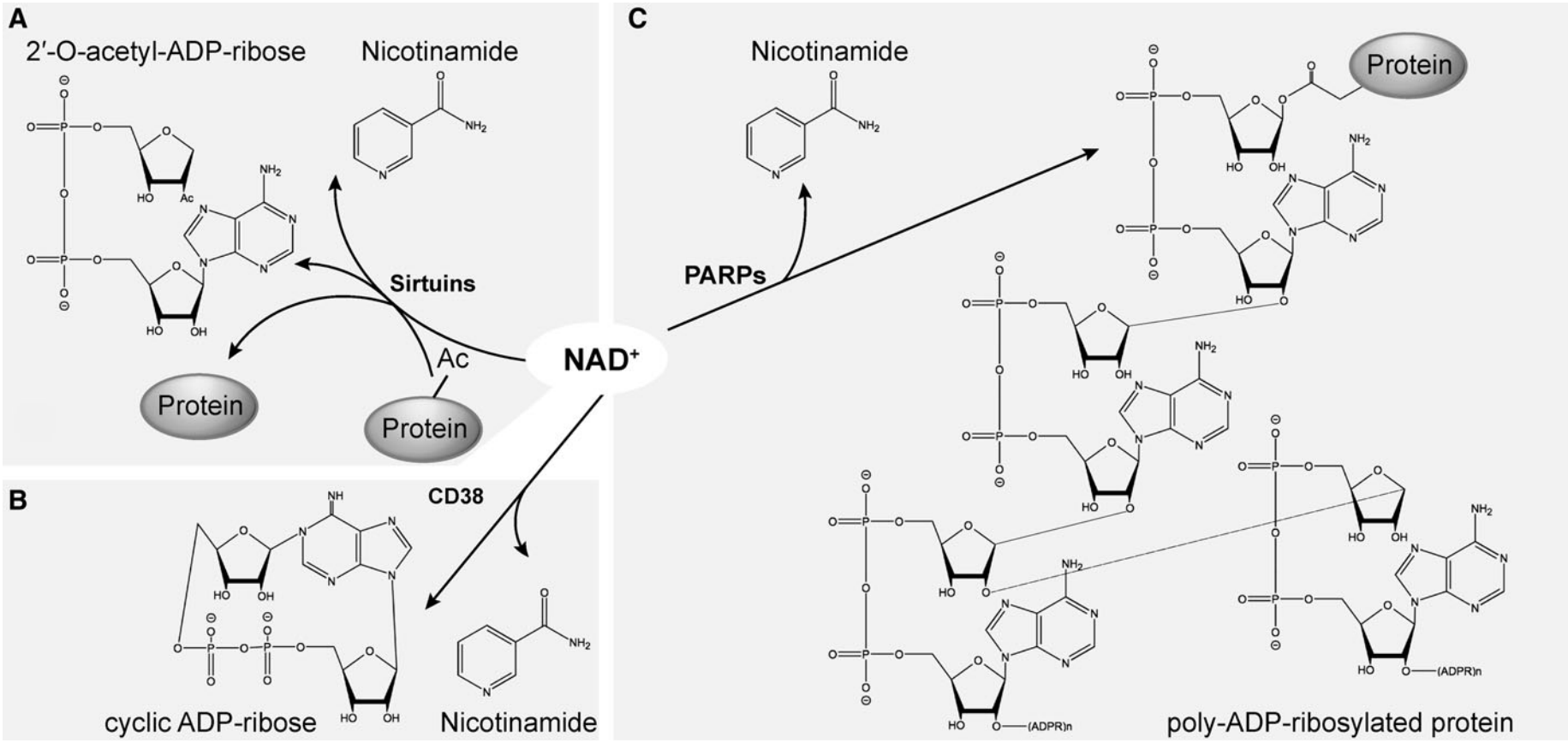

FIG. 2. NAD$^+$-consuming reactions. **(A)** NAD$^+$ hydrolysis is coupled to deacetylation reactions by sirtuins. **(B)** Cyclic ADP-ribose production by ADP-ribosyl cyclases, including CD38 and CD157. **(C)** PARPs catalyze the addition of ADP-ribose to an acceptor protein following with extension and branching of the chain to form poly(ADP-ribose) polymers. Member of this family may also have mono-ADP-ribosylation activity, catalyzing the transfer of a single ADP-ribose moiety to the acceptor protein. All three reactions produce nicotinamide as a by-product. PARP, poly(ADP-ribose) polymerase.

calcium-mobilizing system in neurons (184). Highlighting the array of biological processes in which this enzyme seems to be involved, CD38 signaling has also been shown to mediate mitochondrial transfer from astrocytes to neurons (73).

CD157 also produces cADPR, however, compared to CD38, the catalytic efficiency of CD157 seems to be significantly lower (100). While CD157 has a role in immune development, it has also been recently associated with the development of late-onset PD in specific populations (110, 140, 145). In addition, during development, CD157 expression is observed in nestin-positive cells of the ventricular and subventricular zones, suggesting a yet unrecognized role during neuronal development (38, 75).

Sirtuins

Sirtuins (Sir2-like enzymes) are NAD^+-dependent deacylases that play a key role in transcription, DNA repair, metabolism, and oxidative stress resistance (78). There are seven mammalian sirtuins (SIRT1–7) with diverse subcellular localization, enzymatic activity, and protein substrates (Fig. 3). SIRT1, SIRT6, and SIRT7 localize to the nucleus, SIRT2 is cytoplasmic, and SIRT3, SIRT4, and SIRT5 are present in the mitochondria. Under specific circumstances, SIRT1 and SIRT2 may shuttle between their respective compartments (74).

SIRT4 harbors ADP-ribosyltransferase and lipoamidase activity (70, 105). SIRT5 is an NAD^+-dependent protein lysine demalonylase, deglutarylase, and desuccinylase (53). SIRT6 and SIRT7 display weak deacetylase activity *in vitro* and instead, SIRT6 can catalyze ADP-ribosylation and lysine defatty acylation (56, 80, 93), while SIRT7 function as an NAD^+-dependent histone desuccinylase (92). SIRT1, SIRT2, and SIRT3 are the best-characterized members of the family. They display robust deacetylation activity. In this reaction, the hydrolysis of NAD^+ is coupled to the removal of the acetyl group from the ε-amino group of acetylated lysine residues present in substrate proteins, generating nicotinamide and 2′-O-acetyl-ADP ribose as by-products (57).

It has become evident that lysine acetylation is a prevalent regulatory mechanism of protein function, and thousands of acetylated proteins from different subcellular compartments, including the nucleus, cytoplasm, mitochondria, and endoplasmic reticulum, have been identified by mass spectrometry (43, 99, 129). An estimate of 63% of mitochondrial proteins is subject to reversible lysine acetylation, and NAD^+-dependent regulation of their acetylation status is key for optimal mitochondrial function (13). Although some metabolic enzymes display increased enzymatic activity following acetylation, lysine acetylation negatively affects the activity of most metabolic enzymes (182). Hence, in general, sirtuin-mediated deacetylation is associated with activation of enzymatic processes.

Sirtuins Link NAD^+ Levels to Mitochondrial Function and Antioxidant Defenses

Sirtuins have a significant and ever growing number of protein targets and a comprehensive description of these targets is outside the scope of this review. However, some of these targets have a direct influence on mitochondrial function and antioxidant defenses and exemplify the potential therapeutic role of sirtuins in neurodegenerative diseases.

SIRT1 deacetylates histones at specific gene loci and thereby silences expression of the corresponding genes. In addition to its role as a true histone deacetylase, SIRT1 modulates the activity of several transcription factors and coactivators. In response to increased NAD^+ levels, SIRT1 activates peroxisome proliferator-activated receptor coactivator 1α (PGC-1α) and forkhead box O1 (FOXO1) to increase mitochondrial biogenesis and function (16, 67, 114, 179). SIRT1 can also regulate mitophagy (79, 119) and the mitochondrial unfolded protein response (112), a

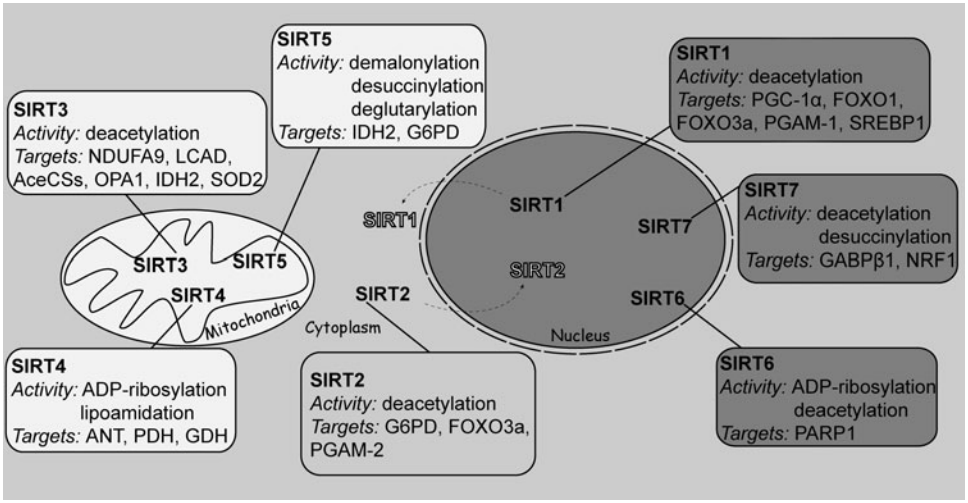

FIG. 3. Subcellular localization, enzymatic activity, and selected substrates of the seven mammalian sirtuins. SIRT1, SIRT6, and SIRT7 localize to the nucleus, SIRT2 is cytoplasmic, and SIRT3, SIRT4, and SIRT5 are mitochondrial. SIRT1 and SIRT2 may shuttle between their respective compartments. See the text for details on activities and substrates. ANT, adenine nucleotide translocator; G6PD, glucose-6-phosphate dehydrogenase; GABPβ1, GA repeat binding protein beta 1; GDH, glutamate dehydrogenase; IDH2, isocitrate dehydrogenase 2; LCAD, long chain acyl-CoA dehydrogenase; NDUFA9, NADH:ubiquinone oxidoreductase subunit A9; NRF, nuclear respiratory factor; OPA1, optic atrophy 1; PDH, pyruvate dehydrogenase; PGAM-1, phosphoglycerate mutase-1; PGC-1α, peroxisome proliferator-activated receptor coactivator-1α; SIRT, sirtuin; SOD, superoxide dismutase; SREBP1, sterol regulatory element binding protein 1.

transcriptional response that promotes cell survival and mitochondrial repair during mitochondrial dysfunction or accumulation of unfolded proteins within the mitochondria (82). Thus, SIRT1 activity affects the overall quantity and quality of mitochondria. Moreover, SIRT1 controls energy production by modulating glucose and lipid homeostasis through direct regulation of phosphoglycerate mutase-1 (PGAM-1) and sterol regulatory element binding protein 1 (SREBP1), respectively (40). SIRT1 may also have a direct role in regulating antioxidant defenses, by deacetylating the transcription factor Nrf2 (nuclear factor, erythroid 2 like 2) (84, 183), a master regulator of antioxidant and phase II enzymes (167). However, the exact biological significance of this observation remains to be elucidated.

All major mitochondrial processes, such as tricarboxylic acid cycle (TCA), fatty acid metabolism, oxidative phosphorylation, mitochondrial acetyl-CoA production, and antioxidant response, are regulated by acetylation. While the mechanisms leading to mitochondrial protein acetylation remain uncertain (120, 149, 172), SIRT3 has clearly emerged as the main deacetylase activity in the mitochondria (95). To date, over 1000 SIRT3 targets have been identified (13), including the 39-kDa NDUFA9 subunit of complex I (2, 34), long chain acyl-CoA dehydrogenase (LCAD), and acetyl-CoA synthases (AceCSs) (71, 76, 148). SIRT3 promotes mitochondrial function not only by regulating the activity of metabolic enzymes but also by regulating mitophagy (180), the mitochondrial unfolded protein response (61), and optic atrophy 1 (OPA1)-dependent mitochondrial dynamics (142).

SIRT3 activity also has a major role in the regulation of mitochondrial antioxidant defenses, by activating mitochondrial superoxide 2 (SOD2) and isocitrate dehydrogenase 2 (IDH2). The activation of SOD2 has a direct effect on superoxide production by the mitochondria. Interestingly, overexpression of SOD2 alone does not reduce the levels of reactive oxygen species to the same extent as its coexpression with SIRT3 does (41, 132, 157). SIRT3-mediated IDH2 activation increases mitochondrial availability of NADPH, which is necessary for the regeneration of reduced glutathione. Reduced glutathione in turn is essential for mitochondrial antioxidant defenses (151, 191). While changes in protein acetylation affect the redox status of the cell, changes in the redox status of the cell can also change the acetylation status of several metabolic and antioxidant pathways, suggesting that these two phenomena are functionally linked (127).

Other members of the sirtuin family also have established and emerging roles regulating mitochondrial function and antioxidant defenses. For example, SIRT2 deacetylates FOXO3a in response to oxidative stress and caloric restriction, and hence increases the expression of SOD2 (173). In addition, SIRT2-mediated deacetylation and activation of glucose-6-phosphate dehydrogenase (G6PD) enhance the activity of the pentose phosphate pathway to supply cytosolic NADPH to counteract oxidative damage (178). Moreover, it has been recently shown that SIRT2 associates with the inner mitochondrial membrane and that loss of SIRT2 leads to increased oxidative stress, decreased ATP levels, and altered mitochondrial morphology in cells from the CNS (94).

Meanwhile, SIRT4-mediated lipoamidation inhibits the activity of pyruvate dehydrogenase (PDH), the enzyme linking glycolysis to the TCA cycle (105). SIRT4 also inhibits glutamate dehydrogenase (GDH) activity, leading to downregulation of insulin secretion in response to amino acids (70). Furthermore, by interacting with the adenine nucleotide translocator (ANT), SIRT4 may regulate the ATP:ADP ratio and membrane potential (3, 77).

SIRT5 activity also has a significant impact in the overall antioxidant defenses. In addition to its role in regulating various metabolic pathways, SIRT5 activates IDH2 through desuccinylation and G6PD through deglutarylation (194). SIRT6 mono-ADP-ribosylates PARP1 and stimulates PARP1-dependent repair of DNA double-strand breaks under oxidative stress (102, 164). Moreover, in a model of cellular senescence, SIRT6 appears to be a new coactivator of Nrf2, being required for the transactivation of Nrf2-driven antioxidant genes (122).

Finally, SIRT7 controls epigenetic and cellular homeostasis through deacetylation of histones and other nuclear proteins. SIRT7 also promotes, through histone desuccinylation, chromatin condensation and DNA double-strand breaks repair in a PARP1-dependent manner (92). Moreover, a significant role for SIRT7 on mitochondrial homeostasis has emerged over the recent years. SIRT7 deacetylates GA repeat binding protein beta 1 (GABPβ1), thus modulating the activity of the GABPα/GABPβ heterotetramer (also known as nuclear respiratory factor or NRF2), a master regulator of nuclear-encoded mitochondrial genes. Consequently, SIRT7 knockout mice display multisystemic mitochondrial dysfunction (139). In addition, SIRT7 can repress NRF1 activity to reduce the expression of the mitochondrial translation machinery, therefore promoting nutritional stress resistance (111).

Interaction Between NAD^+-Consuming Enzymes

The *Km* of sirtuins for NAD^+ seem to be in range with the estimated concentration of NAD^+ in their respective compartments (33, 35). Regardless of whether sirtuins act as *bona fide* NAD^+ sensors, in general the reported *Km* for PARP1 and CD38 are smaller than those of the sirtuins. Thus, PARP1 and CD38 activity limits endogenous sirtuin activation by reducing NAD^+ availability, and inhibition of these main NAD^+ consumers increases NAD^+ levels. It has been shown that decreased PARP1 and CD38 activity effectively increases total NAD^+ content, resulting in SIRT1 activation and protection against the development of metabolic disorders in mice. Protection is conferred through NAD^+-dependent activation of the SIRT1/PGC-1α axis and increased mitochondrial metabolism (4, 16, 17).

Consistently with its rate-limiting role in the NAD^+ salvage pathway, nicotinamide phosphoribosyltransferase (NAMPT) overexpression or its knockdown is associated with increased or reduced SIRT1 activity, respectively (60, 162, 163). Moreover, administration of NAD^+ precursors or a CD38 inhibitor to mice increases NAD^+ content and improves oxidative metabolism thorough an SIRT1/SIRT3-dependent mechanism (34, 54, 187). These early observations lead to the notion that modulating NAD^+ availability, through inhibition of NAD^+-consuming enzymes (PARP1 or CD38) or induction of NAD^+ synthesis, can be a potential therapeutic approach for age-related diseases.

NAD^+ Synthesis

To maintain viability, cells have to continuously synthesize NAD^+. NAD^+ neosynthesis can occur from L-tryptophan

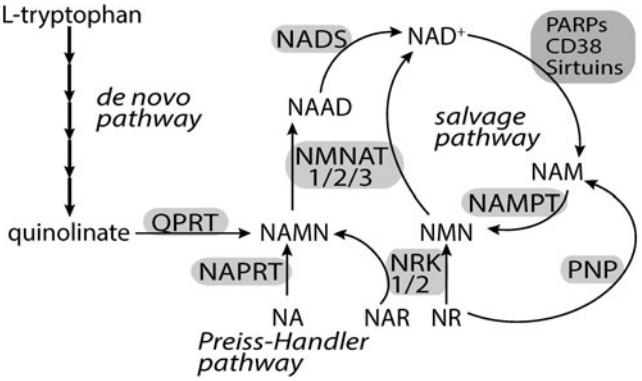

FIG. 4. NAD^+ biosynthetic pathways in mammals. Four building blocks may be used for NAD^+ synthesis: L-tryptophan, NA, NR, and NAM. The *de novo* pathway uses L-tryptophan to generate, through several intermediate steps, quinolinate. Quinolinate is sequentially converted to NAMN, NAAD, and NAD^+ by the action of QPRT, NMNATs, and NADS, respectively. In the Preiss-Handler pathway, NAPRT converts NA to NAMN, which is then used by NMNATs. NR can enter the salvage pathway either by the action of NRK, which generates NMN, or by the reaction catalyzed by PNP, which generates NAM. NAM is subsequently converted to NMN by NAMPT, the rate-limiting step in the salvage pathway. NRKs can also convert NAR to NAMN. All the biosynthetic pathways converge at the level of dinucleotide formation catalyzed by the NMNATs. NMNATs use NAMN and NMN with similar efficiency. NAD^+-consuming reactions catalyzed by PARPs, CD38, and sirtuins release NAM that may be use to resynthesize NAD^+ in the salvage pathway. NA, nicotinic acid; NAD^+, nicotinamide adenine dinucleotide; NAAD, nicotinic acid adenine dinucleotide; NADS, NAD^+ synthetase; NAM, nicotinamide; NAR, nicotinic acid riboside; NR, nicotinamide riboside; NRK, NR kinase; NMN, nicotinamide mononucleotide; NAMN, nicotinic acid mononucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NAPRT, nicotinic acid phosphoribosyltransferase; NMNAT, nicotinamide mononucleotide adenylyl transferase; PNP, purine nucleoside phosphorylase; QPRT, quinolinate phosphoribosyltransferase.

(kynurenine pathway), nicotinic acid (Preiss-Handler pathway), or nicotinamide riboside (NR) (Fig. 4) (20, 23, 138). However, since all the major NAD^+-consuming enzymes generate nicotinamide (NAM) as a by-product, eukaryotic cells have evolved a rescue pathway capable of resynthesizing NAD^+ from NAM. The enzyme NAMPT catalyzes the conversion of NAM and 5′-phosphoribosyl-1-pyrophosphate to NMN; subsequently, nicotinamide mononucleotide adenylyl transferases (NMNATs) transfer adenine from ATP to NMN to generate NAD^+ (48, 62). The relative contribution of the salvage pathway to the overall NAD^+ synthesis rate is exemplified by the observation that homozygous deletion of the *Nampt* gene causes embryonic lethality (137), while deletion of the quinolinate phosphoribosyltransferase (*Qprt*) gene does not (159). All the biosynthetic pathways converge at the level of dinucleotide formation catalyzed by the NMNATs. However, NAMPT is the rate-limiting enzyme in the salvage pathway and overexpression of NAMPT, but not NMNATs, increases cellular NAD^+ levels (117, 136, 186).

Extracellular NMN and NR can serve as NAD^+ precursors and effectively increase total and mitochondrial NAD^+ content in different cell types (152). However, an NMN transporter has not been identified in eukaryotic cells and it appears that the nucleotide is degraded to the nucleoside (NR) to enter the cell (117). The ectoenzyme CD73, a 5′-nucleotidase, has been shown to dephosphorylate NMN to produce NR (69). NR is then imported into the cell by the equilibrative nucleoside transporters (ENTs), and inhibition of ENTs prevents the increase in cellular NAD^+ induced by NMN and NR treatments (117, 135). Once in the cytosol, NR can be phosphorylated by NR kinases (NRKs) to produce NMN (135). Cells are also capable of using NR in an NRK-independent way, in which the purine nucleoside phosphorylase (PNP) generates NAM that enters the salvage pathway (21).

In addition to NR, NRKs can also phosphorylate nicotinic acid riboside (NAR) to produce nicotinic acid mononucleotide (NAMN). NR and NAR can be produced and released by cells, suggesting that one cell may support NAD^+ synthesis in a neighboring cell by providing ribosides as NAD^+ precursors (89). This is an interesting possibility that could have important implications for neuroprotection, similar to the observation that release of glutathione by astrocytes boosts glutathione levels in cocultured neurons (167).

As the relative NAD^+ concentration in different subcellular compartments has a decisive effect on redox and nonredox reactions, the compartmentalization of NAD^+-synthesizing and NAD^+-consuming enzymes has important functional implications (Fig. 5). In general, NAMPT localizes in the cytoplasm and the nucleus and is absent from the mitochondrial fraction (130, 152, 186). In addition, a form of extracellular NAMPT has been described. It is produced by some cell types and seems to have cytokine-like activity (63).

The three different isoforms of NMNAT found in mammalian cells have distinctive subcellular localization. NMNAT1 is found in the nucleus, NMNAT2 is localized in the cytoplasm, and NMNAT3 is found in the mitochondria (22), although this has been recently challenged (58, 185). The relative expression levels of NMNAT3 may influence the role of this enzyme in the modulation of the mitochondrial NAD^+ pool. Thus, in cells that express relatively high levels of NMNAT3, as in HEK293T cells, deletion significantly reduces mitochondrial NAD^+ levels. In contrast, in cells that express NMNAT3 at low levels, as in HeLa cells, its deletion has no effect on the levels of NAD^+ in the organelle (33, 165).

Mitochondrial NAD^+ pool ranges from 40% to 70% of the total NAD^+ content (33, 152). Since NAD^+ is unable to cross the mitochondrial internal membrane, and a mammalian mitochondrial NAD^+ transporter has not yet been identified, the origin of the mitochondrial NAD^+ pool remains to be fully characterized. However, isolated mitochondria can synthesize NAD^+ from NMN (18), and we have recently demonstrated that expression of a mitochondria-targeted NAMPT effectively increases total and mitochondrial NAD^+ levels in astrocytes (72). This observation has several important implications. First, mitochondrial NAD^+ degradation seems to account for a significant portion of cellular NAD^+ turnover, and it is possible to modulate NAD^+ levels by enhancing NAD^+ salvage in a compartment-specific manner. Second, overexpression of NAMPT or the mitochondria-targeted NAMPT increases mitochondrial NAD^+ to similar levels, suggesting that in basal conditions, cytoplasmic NAMPT activity determines mitochondrial NAD^+ levels in astrocytes.

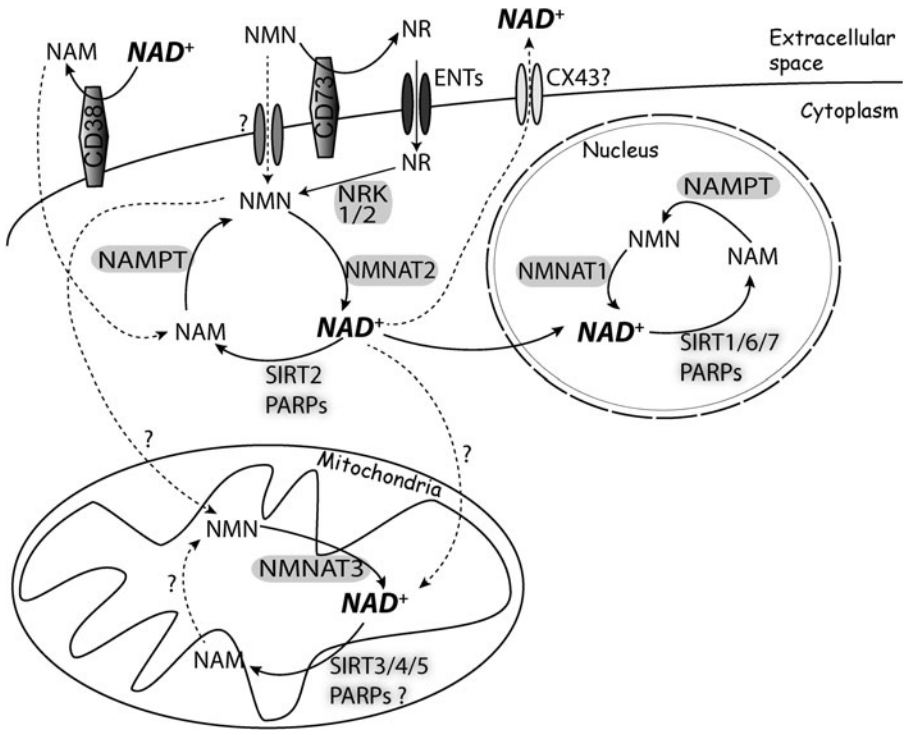

FIG. 5. Compartmentalization of NAD^+ synthesis and utilization. NMN and NR effectively increase total and mitochondrial NAD^+. No NMN or NAD^+ transporters have been identified in mammalian cells. CD73 dephosphorylate NMN to produce NR, which is then imported into the cell, probably by ENTs. Mitochondria can synthesize NAD^+ from NMN, but transport of cytoplasmic NAD^+ into the mitochondria also appears to contribute to the mitochondrial NAD^+ pool. NAD^+ is consumed intracellularly by PARPs and sirtuins, although CD38 has also been found in the endoplasmic reticulum and in the nuclear and mitochondrial membranes (not shown). NAD^+ can also be transported outside of the cell, likely through connexin 43 hemichannels (30). CD38 and CD157 consume extracellular NAD^+. Cells can also synthesize NAD^+ from nicotinic acid and tryptophan (Fig. 4). *Dashed lines* and *question marks* indicate steps for which the exact mechanism has not been fully characterized. ENT, equilibrative nucleoside transporter.

Finally, the data provide additional evidence for the existence of a mitochondrial enzymatic activity capable of using NMN to synthetize NAD^+.

NAD^+ Levels in Aging

Aging is the most important risk factor for the development of many neurodegenerative diseases. It is defined by the reduced ability of the organism to maintain cellular homeostasis over time, leading to accumulated damage to DNA, proteins, membranes, and cellular organelles, including mitochondria. NAD^+ concentration decreases during aging in animal models and in humans (26, 32, 112, 195). *In vivo* imaging data showed for the first time that both NAD^+ levels and mitochondrial function decline in the brain of old healthy humans compared to younger subjects (195). Several mechanisms have been proposed to explain the decline of NAD^+ concentration with aging. NAMPT expression itself may decline with aging, and ablation or overexpression of NAMPT, respectively, aggravates or prevents age-related changes (59, 153, 162). In addition, the decrease of NAD^+ with aging could also be explained by the fact that NAMPT expression displays circadian oscillation; and since circadian oscillation dampens with aging, the overall total levels of NAMPT expression could be affected (39).

On the contrary, increased NAD^+ degradation could also account for the decline in NAD^+ levels observed in aging. As DNA damage accumulates with aging, PARP1 activation may lead to decreased NAD^+ levels and metabolic impairment. However, since PARP1 activation contributes to maintain genomic integrity, the role of PARP1 activation during aging seems to be multifaceted (14). A key role for CD38 in age-related NAD^+ decline has been recently proposed (32). In mice, aging is associated with an increase in CD38 activity, which negatively correlates with NAD^+ levels and mitochondrial activity; an effect attributed at least, in part, to a decrease in SIRT3 activity. In contrast, CD38 knockout mice seem to be protected from this age-related decline in NAD^+ and mitochondrial activity. (32). Since the activity of sirtuins declines with aging (78), these observations link the reduction of NAD^+ levels observed during aging as a leading cause for decreased sirtuin activity and possibly the development of age-related diseases. Accordingly, increasing NAD^+ availability counteracts the effects of aging in many different models (169). Indeed, NAD^+ supplementation has been shown to increase mitochondrial and stem cell function leading to a significant increase in the life span of mice (193).

Circadian Rhythm

The circadian rhythm coordinates metabolism and behavior to recurring daily environmental changes such as light/dark cycles and food availability (147). The ability to

anticipate these changes increases the fitness of the organism, while impairment of normal circadian rhythmicity leads to a range of metabolic defects (12, 19, 107, 154). The levels of NAD^+ display circadian rhythmicity due to direct clock transcriptional control of NAMPT expression (113, 134). In turn, NAD^+ completes a feedback loop by driving the activity of SIRT1, which regulates the activity of the circadian core clock transcription factor heterodimer CLOCK:BMAL1 (113, 134). SIRT6 also participates in circadian regulation by governing CLOCK:BMAL1 recruitment to circadian gene promoters. In addition, SIRT6 controls circadian lipid profiles in the liver by modulating SREBP1 promoter recruitment (104).

Moreover, as mentioned earlier, the activity of key enzymes involved in all major mitochondrial processes is regulated by lysine acetylation, which displays circadian oscillation (103, 115). Since NAD^+ availability regulates the activity of the mitochondrial deacetylase SIRT3, circadian control of NAD^+ availability directly modulates mitochondrial oxidative metabolism and antioxidant defenses (126). Accordingly, PARP1 and CD38 knockout mice display altered circadian metabolic rhythm (11, 141). A growing body of data obtained from both human and animal studies has documented disruption of circadian timekeeping at the physiological, molecular, and behavioral level in AD, PD, and HD (106, 170). A key question that remains unanswered is whether circadian disruption associated with neurodegeneration contributes to altered metabolism and redox homeostasis.

NAD^+ Metabolism and Neurodegeneration

The role of sirtuins in the context of neurodegeneration has been widely studied (51, 74, 78). The following section reviews evidence showing that manipulation of NAD^+ metabolism can be a potential therapeutic target for neurodegeneration. It is worth stressing that alterations in NAD^+ metabolism can potentially affect a number of biological processes in a sirtuin-independent manner. For example, although SIRT3 activity can regulate mitochondria antioxidant defenses, the ratio and concentration of NADH and NAD^+ directly determine the rate of superoxide production by mitochondrial complex I (90, 131). Thus, an increase in NAD^+ availability can directly influence reactive oxygen species production by the mitochondria independently of a sirtuin-mediated increase in antioxidant defenses. As new data emerge on the effect of modulating NAD^+ availability in models of neurodegeneration, it will be important to characterize with genetic and pharmacological approaches whether the beneficial effects are indeed linked to activation of sirtuin family members.

Perhaps the most recognized link between NAD^+ metabolism and neuronal degeneration is the effect of the dominant mutation found in the ''Wallerian degeneration slow'' (Wld^s)-mouse on axonal degeneration. In this mouse model, distal axonal degeneration following sciatic nerve transection is significantly delayed (44, 65). Overexpression of the Wld^s gene seems to be protective in some PD and peripheral neuropathy models but fails to confer protection in ALS and spinal muscular atrophy models (44). The Wld^s gene is a triplication of a chimeric gene that encodes full-length NMNAT1 fused to the first 70 amino acids from the ubiquitin conjugation factor E4 B (UBE4B).

While the exact mechanism is still debated, it is now clear that the protection conferred by the Wld^s gene depends on the presence of NMNAT1 (50, 143). However, maintenance of high levels of NAD^+ may not be critical. For example, the absence of PARP1 and CD38 does not confer protection against axonal degeneration despite the observed elevated NAD^+ levels in these models (144). Instead, since axonal injury leads to rapid NMNAT2 depletion, the concomitant accumulation of NMN may be responsible for promoting axonal degeneration (50, 97, 144). Accordingly, reducing NMN levels delays Wallerian degeneration after axonal injury (49, 50, 143, 144). On the contrary, NAD^+ degradation has also been directly linked to axonal degeneration. SARM1 (sterile alpha and TIR motif containing 1) is a central player in the degenerative program activated following axonal injury. SARM1 initiates a local destruction program through a process that involves the catastrophic depletion of axonal NAD^+ (55, 64, 118, 143). Thus, the therapeutic potential of modulating NAD^+ availability in the context of Wallerian degeneration remains to be settled.

In addition, it has been shown that NMNAT2 constitutes an essential survival factor for maintenance of healthy axons, and reduction of NMNAT2 expression below a threshold level triggers degeneration even in uninjured axons (66). Moreover, NMNAT overexpression provides neuroprotection in several models of neurodegeneration (8, 44). In addition to help maintain NAD^+ levels, NMNATs can act as chaperones, a function that is believed to be responsible for the protection observed in some models of proteotoxicity (7, 192). Consistently with its rate-limiting role in NAD^+ synthesis, NAMPT overexpression is also protective in several models of neurodegeneration (72, 81, 176). Interestingly, aminopropyl carbazole derivatives, previously shown to be

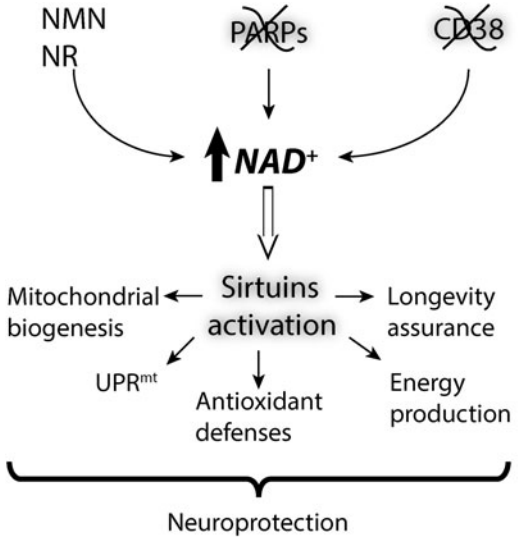

FIG. 6. Possible protective mechanisms responsible for the neuroprotection conferred by increasing NAD^+ availability. Supplementation with NAD^+ precursors/intermediates (NMN and NR) or inhibition of NAD^+-consuming enzymes (PARP1 and CD38) leads to increased NAD^+ availability and sirtuin activity. PARP1 inhibition could have additional effects not depicted in the figure (see NAD^+-dependent signaling section for details).

neuroprotective in ALS and PD models, have been recently identified as NAMPT activators (47, 160, 174).

Two main strategies have arisen to increase NAD^+ availability: (i) inhibition of NAD^+-consuming enzymes and (ii) utilization of bioavailable NAD^+ intermediates (Fig. 6).

PARP inhibition

Excessive PARP activation depletes NAD^+ and impairs mitochondrial function leading to cell death (14). PARP inhibition improves axonal regeneration in *Caenorhabditis elegans* neurons and adult dorsal root ganglia neuronal cultures (27, 31). Interestingly, despite elevated neuronal NAD^+ levels in $PARP1^{(-/-)}$ and $CD38^{(-/-)}$ mice, these deletions confer no protection against sciatic nerve transection (144). PARP1 activity appears to be upregulated in the CNS of AD, HD, and ALS patients, and PARP1 activation leads to progressive loss of dopaminergic neurons in PD models (86, 91, 98, 101, 171).

$PARP1^{(-/-)}$ mice are resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 6-hydroxydopamie toxicity (87, 101), suggesting that PARP1 inhibitors may be beneficial in the treatment of PD. Moreover, PARP inhibitors prevent mitochondrial membrane depolarization and neuronal death in astrocyte/neuronal cocultures exposed to amyloid β (1). Likewise, PARP1 knockout is protective against amyloid β-induced microglial activation and neurotoxicity (83). PARP1 inhibition is also protective in a mouse model of HD (R6/2), but PARP1 knockout aggravates onset and progression in a model of experimental autoimmune encephalomyelitis (37, 150). Comparably, one of the earliest developed PARP inhibitors failed to confer protection in an ALS animal model (10).

Since in neurodegenerative processes dying cells are exposed to increased oxidative stress, and PARPs play a central role in DNA damage repair, further work is needed to establish the value of long-term PARP1 inhibition as a therapeutic target in neurodegenerative diseases. It is worth stressing that in addition to prevent NAD^+ depletion due to PARP1 overactivation, PARP1 inhibition will also prevent poly(ADP-ribose)-dependent deleterious effects (see NAD^+-dependent signaling section). Thus, the relative contribution of these two components to the pathogenic process may differentially affect the outcome of PARP inhibition in different diseases.

CD38 inhibition

Ablation of CD38 results in a significant increase in the steady-state levels of NAD^+ in the brain; with reported changes ranging from 2- to 10-fold increases (5, 188). All studies to date indicate that CD38 activity is constitutive, but can be upregulated following CNS injury. In focal cerebral ischemia, CD38 is upregulated, and $CD38^{(-/-)}$ mice display reduced cerebral injury and immune cell infiltration after ischemia (42, 88). Consistently with its role in the immune system (100), CD38 is likely to have a significant part in the activation of astrocytes and microglia. However, relative scarce data exist about the potential role of CD38 during chronic neurodegeneration. CD38 regulates macrophages and microglia migration to inflammatory mediators such as amyloid β (123). Knocking out CD38 significantly reduces amyloid burden and learning deficits in an AD mouse model (APP/PS) (24). Since CD38 plays a role in many complex biological processes such as stem cell differentiation, transfer of mitochondria between cells, and animal behavior (73, 85, 181), its inhibition can potentially affect neurodegenerative processes in hard to predict ways.

Precursor supplementation

Use of NAD^+ biosynthetic intermediates/precursors (mainly NMN and NR) has proven to be an effective way to increase NAD^+ availability and sirtuin activity *in vitro* and *in vivo* (35, 78, 152). Much of the available literature on precursor supplementation focuses on the capacity to enhance oxidative metabolism and protect against metabolic disease, but some examples have started to emerge in models of neurodegeneration. NMN administration restores mitochondrial function and cognition in AD animal models (96, 175). In addition, long-term NMN administration (12 months) mitigates age-associated physiological decline in wild-type mice, without any obvious toxicity or deleterious effects (109), highlighting the therapeutic potential of this strategy.

As mentioned earlier, NR is a naturally occurring NAD^+ precursor that can be imported into the cell, where it is phosphorylated by NRKs to produce NMN (117, 135). NR has recently been shown to be orally available and to safely boost NAD^+ levels in mice and humans (161). Dietary supplementation with NR promotes PGC-1α expression and reduction on amyloid β production in an AD mouse model (Tg2576) (68). Moreover, NR administration prevents noise-induced spiral ganglia neurite degeneration in an SIRT3-dependent manner (28).

Using a coculture model, we have shown that boosting NAD^+ specifically in astrocytes could be a valid therapeutic approach to confer neuroprotection. Mouse astrocytes expressing ALS-linked SOD1 mutations and human astrocytes isolated from familial or sporadic ALS cases are toxic to cocultured motor neurons (158). Although there is no consensus on the nature of the toxic mediator, increasing antioxidant defenses in ALS astrocytes reverts this neurotoxic phenotype (128, 166, 168). Treatment of ALS astrocytes with NMN or NR decreases the production of reactive oxygen species by the mitochondria and rescues the toxicity toward motor neuron (72). Thus, increasing NAD^+ availability may be a viable therapeutic approach to prevent astrocyte-mediated motor neuron death in ALS.

Conclusions

It has become clear that NAD^+-dependent signaling has a major impact in many biological processes. Particularly, its manipulation can provide protection against pathologies linked to mitochondrial dysfunction and oxidative stress. Even though the detailed mechanism responsible for the observed protection is not yet established, increasing NAD^+ availability is neuroprotective in several *in vitro* and *in vivo* models. The increase in NAD^+ availability is likely to increase endogenous sirtuin activity and offset NAD^+ depletion caused by PARP or CD38 overactivation. The emerging protective mechanism will most likely be relevant in the context of several, acute or chronic, pathological conditions of the CNS. Thus, therapies targeting NAD^+ availability could contribute to the development of integral approaches

for the treatment of ischemic injury, trauma, and chronic neurodegenerative diseases.

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Address correspondence to:
Dr. Marcelo R. Vargas
Department of Cell and Molecular Pharmacology and Experimental Therapeutics
Medical University of South Carolina
Basic Science Building, Room 358
MSC 509, 173 Ashley Avenue
Charleston, SC 29425

E-mail: vargasm@musc.edu

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Abbreviations Used

AceCSs = acetyl-CoA synthases
AD = Alzheimer's disease
ALS = amyotrophic lateral sclerosis
ANT = adenine nucleotide translocator
BMAL1 = brain and muscle ARNT-Like 1
cADPR = cyclic ADP ribose
CD38 = CD38 antigen
CLOCK = circadian locomotor output cycles kaput
CNS = central nervous system
ENTs = equilibrative nucleoside transporters
FOXO1 = forkhead box O1
FOXO3a = forkhead box O3a
G6PD = glucose-6-phosphate dehydrogenase
GABPα = GA repeat binding protein, alpha
GABPβ1 = GA repeat binding protein, beta 1
GDH = glutamate dehydrogenase
HD = Huntington's disease
IDH2 = isocitrate dehydrogenase 2
LCAD = long chain acyl-CoA dehydrogenase
NAAD = nicotinic acid adenine dinucleotide
NAD^+ = nicotinamide adenine dinucleotide
NADH = nicotinamide adenine dinucleotide (reduced)
NADS = NAD^+ synthetase
NAM = nicotinamide
NAMN = nicotinic acid mononucleotide
NAMPT = nicotinamide phosphoribosyltransferase
NAPRT = nicotinic acid phosphoribosyltransferase
NAR = nicotinic acid riboside
NDUFA9 = NADH:ubiquinone oxidoreductase subunit A9
NMN = nicotinamide mononucleotide
NMNAT = nicotinamide mononucleotide adenylyl transferase
NR = nicotinamide riboside
NRF = nuclear respiratory factor
Nrf2 = nuclear factor, erythroid 2 like 2
NRK = NR kinase
OPA1 = optic atrophy 1
PARP = poly(ADP-ribose) polymerase
PD = Parkinson's disease
PDH = pyruvate dehydrogenase
PGAM-1 = phosphoglycerate mutase-1
PGC-1α = peroxisome proliferator-activated receptor coactivator-1α
PNP = purine nucleoside phosphorylase
QPRT = quinolinate phosphoribosyltransferase
SARM1 = sterile alpha and TIR motif containing 1
SIRT = sirtuin
SOD = superoxide dismutase
SREBP1 = sterol regulatory element binding protein 1
TCA = tricarboxylic acid cycle
UBE4B = ubiquitin conjugation factor E4 B
Wld^s = Wallerian degeneration slow