REVIEW

NAD$^+$ in aging, metabolism, and neurodegeneration

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Nicotinamide adenine dinucleotide (NAD$^+$) is a coenzyme found in all living cells. It serves both as a critical coenzyme for enzymes that fuel reduction-oxidation reactions, carrying electrons from one reaction to another, and as a cosubstrate for other enzymes such as the sirtuins and poly(adenosine diphosphate–ribose) polymerases. Cellular NAD$^+$ concentrations change during aging, and modulation of NAD$^+$ usage or production can prolong both health span and life span. Here we review factors that regulate NAD$^+$ and discuss how supplementation with NAD$^+$ precursors may represent a new therapeutic opportunity for aging and its associated disorders, particularly neurodegenerative diseases.

Nicotinamide adenine dinucleotide (NAD$^+$) is a key cellular factor for intermediary metabolism. Originally defined as a molecular fraction ("cozymase") that accelerated fermentation in yeast extracts, its chemical structure was resolved as a nucleotide sugar phosphate. The nicotinamide portion of NAD$^+$ is the site of reduction-oxidation (redox) reactions, and its reduced form (NADH) serves as a key energy-transfer intermediate between different metabolic pathways (Fig. 1).

The medical importance of NAD$^+$ was established early with the discovery of pellagra, a disease characterized by four "Ds": dermatitis, diarrhea, dementia, and death. A heat-stable dietary factor (known as pellagra-preventing factor) that cured pellagra was determined to be a NAD$^+$ precursor called niacin. This provided the first evidence of a therapeutic role for what is now vitamin B_3 (*1*).

Although pellagra is rare in the developed world, decreased cellular NAD$^+$ concentrations occur under defined conditions, including aging, and supplementation with NAD$^+$ precursors may be useful against aging and its chronic diseases. Here we review recent findings on NAD$^+$ biology and their implications for normal aging and age-associated diseases.

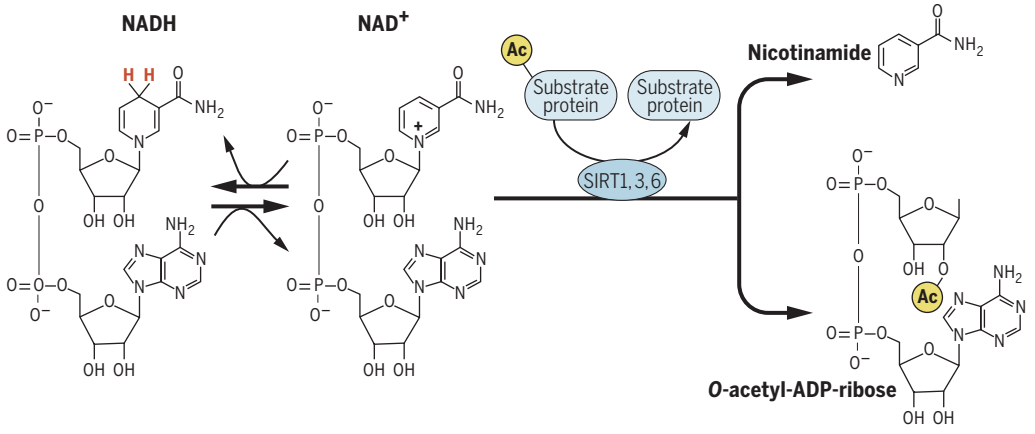

Fig. 1. NAD$^+$ can be used as both a cosubstrate and a coenzyme. The structures of NAD in its reduced (NADH) and oxidized (NAD$^+$) forms are shown (hydrides in red). Sirtuins remove acetyl and acyl groups from target proteins. This reaction is coupled to NAD$^+$ cleavage into nicotinamide, and the ADP-ribose serves as the acceptor for the removed acetyl (or acyl) group (in yellow).

NAD$^+$ biosynthesis, degradation, and salvage

Beyond its role as a coenzyme in redox reactions, NAD$^+$ is an important cosubstrate for three classes of enzymes: (i) the sirtuins (SIRTs), (ii) the adenosine diphosphate (ADP)–ribose transferases (ARTs) and poly(ADP-ribose) polymerases (PARPs), and (iii) the cyclic ADP-ribose (cADPR) synthases (CD38 and CD157). NAD$^+$ is consumed by these enzymes and continuously degraded (Fig. 1). To maintain stable cellular concentrations of NAD$^+$, organisms primarily use a nicotinamide salvage pathway but also rely on several biosynthetic pathways.

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NAD$^+$ biosynthetic pathways

Nicotinamide adenine dinucleotide can be synthesized from diverse dietary sources, including nicotinic acid and nicotinamide, tryptophan, and nicotinamide riboside (NR). The major dietary source of NAD$^+$ is nicotinic acid, a form of niacin (i.e., vitamin B_3) that can be transformed into NAD$^+$ through three steps in the Preiss-Handler pathway (Fig. 2). A key enzyme in this pathway is nicotinamide mononucleotide adenylyltransferase (NMNAT), which transforms nicotinic acid mononucleotide (NAMN) into nicotinic acid adenine dinucleotide (NAAD) in the presence of adenosine triphosphate (ATP). Three forms of the enzyme have distinct subcellular localizations: NMNAT1 in the nucleus, NMNAT2 in the cytosol and Golgi, and NMNAT3 in the cytosol and mitochondria (*2*–*4*). This enzyme is also important in the NAD$^+$ salvage pathway.

Synthesis of NAD$^+$ from tryptophan occurs in the kinurenine pathway (*5*) (Fig. 2). The first step is conversion of tryptophan to *N*-formylkinurenine by indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO). IDO and TDO activities lead to metabolites in the kinurenine pathway that modulate the activity of the mammalian immune, reproductive, and central nervous systems. This is a rate-limiting step in the pathway, and both enzymes are frequently overexpressed in cancer and may contribute to immune tolerance to cancer cells through their immunomodulatory activities. Another key step is transformation of 2-amino-3-carboxymuconate semialdehyde (ACMS). This compound spontaneously condenses and rearranges into quinolinate, which serves as a precursor to NAD$^+$ synthesis (Fig. 2). However, under most circumstances, ACMS is decarboxylated by ACMS decarboxylase into 2-amino-3-muconate semialdehyde (AMS), leading to its oxidation into acetyl–coenzyme A (CoA) via the tricarboxylic acid (TCA) cycle. If the ACMS decarboxylase enzymatic capacity is exceeded by an excess of tryptophan (*6*), quinolinate is transformed into NAMN, thus linking with the Preiss-Handler pathway (Fig. 2).

NAD$^+$ salvage pathway

This is the key pathway for maintaining cellular NAD$^+$ levels. The NAD$^+$-consuming enzymes—the SIRTs, ARTs, and PARPs—all generate nicotinamide as a by-product of their enzymatic activities. Nicotinamide regulates their activities as an inhibitory factor by binding in a conserved NAD$^+$ pocket and also as a biosynthetic precursor to NAD$^+$ via activity of nicotinamide phosphoribosyltransferase (NAMPT). This enzyme recycles nicotinamide into nicotinamide mononucleotide (NMN), which is converted into NAD$^+$ by the various NMNATs (discussed above). This pathway leads to recycling of nicotinamide into NAD$^+$ and relieves nicotinamide inhibition of NAD$^+$-consuming enzymes. NAMPT is expressed in low amounts in pancreatic β cells and neurons, which might allow it to become more rapidly limiting in these cells (*7*). NAMPT can be either intracellular (iNAMPT) or extracellular (eNAMPT).

NAD$^+$ as an enzyme cosubstrate

Nicotinamide adenine dinucleotide is a critical cofactor for other enzymes, including the sirtuin protein deacetylases, the ADP-ribose transferases and PARP, and the cADPR synthases (CD38 and CD157).

The sirtuin protein deacylases

These proteins are conserved from bacteria to humans. They remove acyl groups from lysine residues on proteins in a NAD-dependent manner. NAD$^+$ is cleaved between nicotinamide and ADP-ribose, and the latter serves as an acyl acceptor, generating acyl-ADP-ribose (Fig. 1). The demonstration that Sir2 is an NAD$^+$-dependent protein deacetylase (*8*) suggested that Sir2 enzymatic activity could be coupled to metabolic status, a model supported by the observation that, in *Saccharomyces cerevisiae*, Sir2 is necessary for life-span extension in response to calorie restriction (*9*).

Sirtuins sense intracellular NAD$^+$ concentrations and transduce a signal via protein deacylation, predominantly modifying acetyl but also succinyl, malonyl, glutaryl, palmitoyl, and other fatty acids (*10*, *11*). Yeast sirtuin Sir2 was identified as a gene controlling aging in yeast, and this function was confirmed in worms and *Drosophila melanogaster* (*12*–*14*). After questions were raised as to the robustness of these effects and confounding variables in the genetic background of the strains used (*15*), follow-up studies have confirmed the life-span–extending effects of Sir2 orthologs in flies and worms (*16*–*18*) and for SIRT1 and SIRT6 in mice (*19*, *20*).

There are seven sirtuins in mammals in different subcellular compartments: nuclear for SIRT1, SIRT6, and SIRT7; cytoplasmic for SIRT2; and mitochondrial for SIRT3, SIRT4, and SIRT5. The possibility that NAD$^+$ concentrations are regulated semi-independently in different cellular compartments could allow local changes in NAD$^+$ concentrations to differentially affect the activity of distinct sirtuins.

Calorie restriction, a decrease in caloric intake (by 10 to 40%) without malnutrition, has been shown to increase life span and health span in all organisms in which it has been tested. The benefits of calorie restriction on metabolism and other cellular functions, such as cognition, depend on NAD$^+$ sensing by SIRT1. The protein deacetylase of SIRT1 functions as an epigenetic regulator by targeting specific histone-acetylated residues (e.g., H3K9, H3K14, and H4K16) but also regulates transcription by deacetylating transcription factors (such as TP53, NF-κB, PGC-1α, and FOXO3a) (*21*). As discussed above, NAD$^+$ concentrations fluctuate in a circadian manner and thereby link the peripheral clock to the transcriptional regulation of metabolism by epigenetic mechanisms through SIRT1. The core components of the circadian clock, BMAL1 and CLOCK, directly regulate expression of NAMPT in the NAD$^+$ salvage pathway in mice. SIRT1 protein abundance is relatively stable, but its deacetylase activity depends on NAMPT to generate NAD$^+$, and SIRT1 enzymatic activity oscillations correlate with the circadian production of NAD$^+$. Key targets of SIRT1 include lysines 9 and 14 of histone 3 at multiple loci of genes that oscillate under circadian control (*22*). SIRT1 also regulates ribosomal biogenesis, an energy-consuming process in eukaryotes, particularly in proliferative tissues. SIRT1 is presumably activated through increased levels of NAD$^+$ during the transition to glucose starvation, and it deacetylates histone H3K9 at ribosomal DNA loci, in cooperation with a protein complex that contains nucleomethylin, the heterochromatin methyltransferase SUV39H1, and SIRT1 (*23*).

Sirtuin 1 is phosphorylated by protein kinase A as a consequence of adrenergic receptor activation during fasting. This induces a shift in its Michaelis constant (K_m) for NAD$^+$ that sensitizes the enzyme for fluctuations in NAD$^+$ concentrations (*24*). SIRT6, which is also localized in the nucleus, is linked to aging by regulating telomere stability and inflammation through NF-κB signaling. Deacetylation of histone H3K9 appears to be the modification connecting SIRT6 activity with these aging pathways. Loss of SIRT6 leads to progeria, whereas gain of function extends life span in male mice by 15% (*19*, *25*–*27*).

Sirtuin 3 is the major mitochondrial protein deacetylase (*28*). Its expression is enhanced by fasting and calorie restriction and is decreased during aging and by a high-fat diet (*29*). SIRT3 deacetylates and thereby increases the enzymatic activity of key mitochondrial proteins involved in the protection against oxidative stress and in intermediary metabolic pathways, such as fatty acid oxidation, the urea cycle, and oxidative phosphorylation (*30*–*35*). SIRT3 is necessary for the protective effect of calorie restriction on age-associated hearing loss (*30*). Without SIRT3, mice develop a syndrome similar to the metabolic syndrome in humans, which results in glucose intolerance, mild obesity, chronic inflammation, dyslipidemia, and steatohepatitis (*29*). Mitochondrial NAD$^+$ concentrations fluctuate in a diurnal manner and mediate a change in mitochondrial oxidative activity in a SIRT3-dependent manner (*36*).

In worms, nicotinamide is metabolized into 1-methylnicotinamide (MNA) by a nicotinamide-*N*-methyltransferase (anmt-1). Loss of anmt-1 suppresses the life-span effect of Sir2.1. Thus, Sir2.1 might increase life span by generating nicotinamide, which is then transformed into MNA. MNA serves as a substrate for the aldehyde oxidase

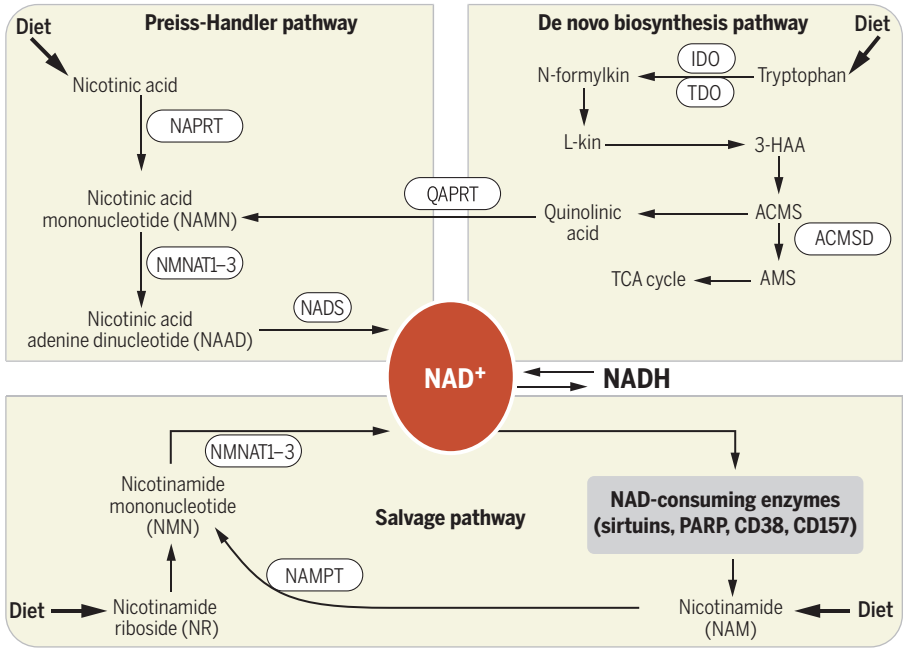

Fig. 2. NAD$^+$ biosynthetic pathways. NAD$^+$ levels are maintained by three independent pathways. First, the Preiss-Handler pathway uses dietary nicotinic acid and the enzyme nicotinic acid phosphoribosyltransferase (NAPRT) to generate NAMN, which is then transformed into NAAD by NAMN transferase (NMNAT). Three forms of this enzyme (NMNAT1, -2, and -3) have distinct subcellular localizations. The process is completed by the transformation of NAAD into NAD$^+$ by NAD$^+$ synthase (NADS). Second, the de novo synthesis pathway of NAD from tryptophan occurs through the kinurenine pathway (*5*). The first step in this pathway is the rate-limiting conversion of tryptophan to *N*-formylkinurenine (*N*-formylkin) by either IDO or TDO. Formylkinurenine is transformed into L-kinurenine (L-kin), 3-hydroxykinurenine, and 3-hydroxyanthranilic acid (3-HAA) and finally to ACMS. This compound can spontaneously condense and rearrange into quinolinic acid, which is transformed into NAMN, at which point it converges with the Preiss-Handler pathway. ACMS can also be decarboxylated into AMS by ACMS decarboxylase (ACMSD), leading to its oxidation into acetyl-CoA via the TCA cycle. Third, the NAD$^+$ salvage pathway recycles the nicotinamide generated as a by-product of the enzymatic activities of NAD$^+$-consuming enzymes: sirtuins, PARPs, and the cADPR synthases (CD38 and CD157). Initially, NAMPT recycles nicotinamide into NMN, which is then converted into NAD$^+$ via the different NMNATs.

GAD-3 to generate hydrogen peroxide, which may promote a mitohormesis response, in which a mild stress in mitochondria promotes a protective response in the whole cell (*37*, *38*). The implications for the mammalian NAD^+-sirtuin-aging link and its relevance to mammalian systems are not clear but warrant further study.

Poly(ADP-ribose) polymerases

There are 17 genes encoding PARP-related proteins (*39*). Activated PARP1 and PARP2 catalyze the transfer of multiple ADP-ribose moieties from NAD^+ to protein acceptors, generating long poly(ADP-ribose) (PAR) chains. Other PARPs are enzymatically inactive or catalyze the transfer of mono-ADP ribose to acceptor proteins and are less important for modulating cellular NAD^+ concentrations. PARP1 is the most abundant PARP and is expressed ubiquitously. This DNA-dependent nuclear PARP is strongly activated by DNA damage, leading to consumption of a large amount of cellular NAD^+. In fact, DNA damage leads to a decrease (up to 80%) in cellular NAD^+ concentrations. PARP1 is important in DNA damage detection and repair, as well as in a cell's decision to repair itself or die after a genotoxic insult (*40*). PARP1 also promotes ribosomal RNA biogenesis by PARylation of several nucleolar proteins and is required for assembly of cytoplasmic stress granules that regulate the stability and translation of mRNA in response to stress (*41*).

Selective PARP inhibitors are in development or are already approved for cancer treatment. They show synthetic lethality with defects in homologous replication or are tested in combination therapy with chemotherapy or radiation therapy (*42*). These inhibitors are also being tested as anti-inflammatory drugs in stroke and myocardial infarction.

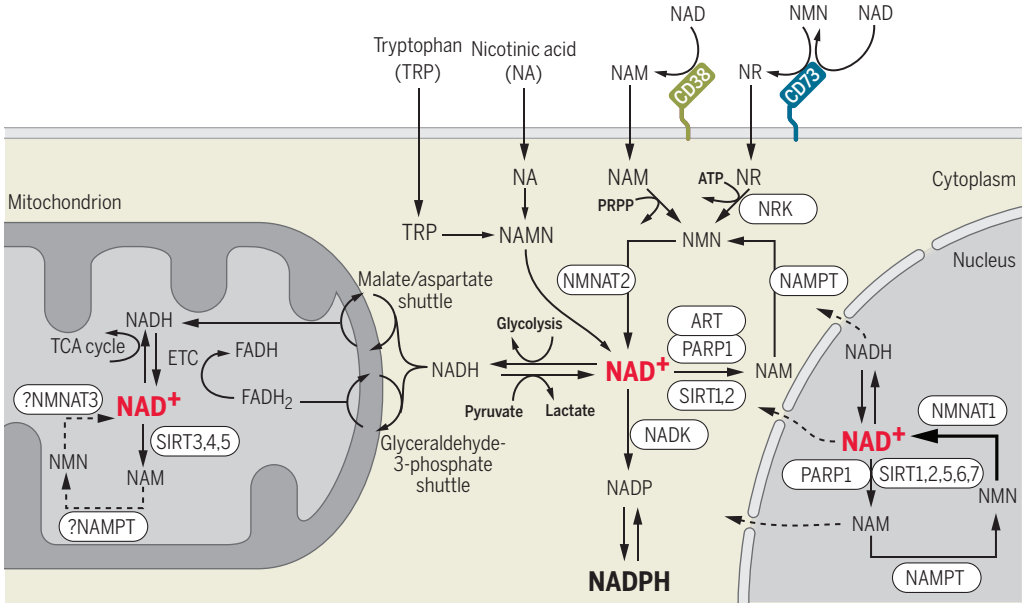

Fig. 3. NAD^+ metabolism in different cellular compartments. The different precursors to intracellular NAD metabolism—tryptophan, nicotinic acid (NA), nicotinamide, NR, and NMN—are shown, along with their extracellular metabolism by CD38 and CD73. The cytoplasmic and nuclear NAD^+ pools probably equilibrate by diffusion through the nuclear pore. However, the mitochondrial membrane is impermeable to both NAD^+ and NADH. Reducing equivalents generated by glycolysis are transferred to the mitochondrial matrix via the malate/aspartate shuttle and the glyceraldehyde-3-phosphate shuttle. The resulting mitochondrial NADH (malate/aspartate shuttle) is oxidized by complex I in the respiratory chain (ETC), whereas the resulting $FADH_2$ (glyceraldehyde-3-phosphate shuttle) is oxidized by complex II. In each of the three compartments, different NAD^+-consuming enzymes lead to the generation of nicotinamide, which is recycled via the NAD^+ salvage pathway. Different forms of the NMNAT enzyme and sirtuins are localized in different compartments. The nature of the salvage pathway for NAD^+ in mitochondria has not been fully resolved, although NMNAT3 has been found in mitochondria. NADK, NAD^+ kinase.

NAD^+ as an enzyme cofactor

Nicotinamide adenine dinucleotide and its phosphorylated derivative, nicotinamide adenine dinucleotide phosphate (NADP), serve as essential coenzymes for hydride-transfer enzymes (Fig. 3). They participate in redox reactions as hydride acceptors (NAD^+ and $NADP^+$) or donors [NADH or NADPH (reduced forms)]. These two redox pairs are kept in chemical opposition: NAD^+ is mostly maintained in its oxidized form; NADP is mostly in its reduced form, NADPH. As a coenzyme, NAD^+ is essential for energy generation by transferring reducing equivalents from glycolysis (from the activity of glyceraldehyde-3-phosphate dehydrogenase) and from the TCA cycle under the form of NADH. When oxygen is limiting, NADH is converted to NAD^+ by reduction of pyruvate into lactate. With oxygen, cytoplasmic NADH transfers its reducing equivalent through the malate-aspartate shuttle or the glycerol-3-3-phosphate shuttle to the mitochondrial matrix (Fig. 3). These reducing equivalents are oxidized by complex I of the electron-transport chain (ETC), thereby coupling glycolysis and the TCA cycle to ATP synthesis via oxidative phosphorylation. NADP is critical in several pathways, including fatty acid oxidation and cholesterol synthesis, as well as in redox protection. In these reactions, NAD^+ and NADH (or NADP and NADPH) interconvert but are not consumed.

Deterioration of mitochondrial function and the accompanying cellular energy deficit have emerged as critical factors in aging and diseases of aging (particularly neurodegenerative diseases). A number of pathways that extend life span help to maintain mitochondrial function, including mitochondrial biogenesis (*43*). The critical role of NAD^+ in mitochondrial intermediary metabolism suggests that variations in NAD^+ concentrations in this compartment may affect metabolic efficiency, aging, and aging-associated diseases. This model is supported by the observation that components of the malate-aspartate NADH shuttle, which transfers reducing equivalent from NADH in the cytoplasm to NADH in the mitochondrial matrix, are required for lifespan extension in response to dietary restriction in yeast (*44*).

Competition between NAD^+-consuming enzymes for shared NAD^+ pools

Although three classes of enzymes use NAD^+, they have distinct roles in aging. Sirtuins are emerging as key mediators of increased life span, and PARPs and NAD^+ hydrolases exhibit the opposite effect. The evidence supporting this emerging model is detailed below (Fig. 4). An interesting competitive relationship exists between SIRT1 and PARP1 or CD38 for NAD^+ (*45*). For example, PARP1 activity increases during aging, as DNA damage accumulates, and in response to high-energy intake (*46*–*48*). Because PARP1 and SIRT1 have relatively similar values of K_m for NAD [50 to 97 μM for PARP1 versus 94 to 96 μM for SIRT1 (*24*, *49*–*52*)], the decrease in NAD^+ concentrations that occurs when PARP1 is activated (up to an 80% decrease, starting from 200 to 500 μM under basal conditions) leads to a decrease in SIRT1 activity.

The antagonistic relationship between SIRT1 and PARP1 goes beyond competition for NAD: SIRT1 also inhibits PARP1 through deacetylation and at the transcriptional level (*53*, *54*), and their activities on key cellular proteins are opposing. For example, activity of the transcription factor NF-κB is suppressed by SIRT1-mediated deacetylation of its component p65/RelaA, whereas NF-κB is transcriptionally activated by PARP1. In the case of tumor protein p53, it is activated by PARP1 under conditions of genotoxic stress

and inactivated by SIRT1 through deacetylation (*55*–*57*).

An extreme example of PARP1 activation occurs in patients with xeroderma pigmentosum group A (XPA), a nucleotide excision DNA repair disorder with severe neurodegeneration. The clinical neurological features of XPA (progressive cerebellar degeneration, peripheral neuropathy, and sensorineural hearing loss) are similar to those in two other DNA damage disorders: ataxia telangiectasia (AT) and Cockayne syndrome (CS). Further, hierarchical clustering of primary mitochondrial diseases with DNA repair disorders based on shared symptoms and manifestations shows a clustering of these DNA damage disorders (CS, XPA, AT) with several primary mitochondrial disorders [such as MEGDEL syndrome, Charcot-Marie-Tooth disease type 2A2, and NARP (neuropathy, ataxia, and retinitis pigmentosa) syndrome] and also with Friedreich ataxia, a disorder associated with defective mitochondrial SIRT3 activity, suggesting a shared pathogenic mechanism, perhaps driven by NAD^+ depletion (*58*, *59*).

Patients with XPA show PARP1 hyperactivation, NAD^+ depletion, and decreased SIRT1 activity (Fig. 4). They also show mitochondrial abnormalities, including decreased mitochondrial autophagy (mitophagy), presumably as a consequence of decreased activity of the NAD^+–SIRT1–PGC-1α axis (*60*). This mitophagy defect in XPA, CS, and AT can be suppressed by treatment with PARP inhibitors or by supplementation with NAD^+ precursors, such as NMN and NR (*60*). This concept was validated in a mouse CS model ($csb^{m/m}$) that carries a mutation in the ortholog of human Cockayne syndrome B, csb. These mice display features characteristic of human Cockayne syndrome—for instance, decreased life span, mitochondrial abnormalities (hyperpolarized, increased O_2 consumption), and neuronal damage in the cerebellum and inner ear. As with XPA, PARP inhibitors or NAD^+ precursors suppressed these phenotypes (*61*). A high-fat diet associated with a small increase in the ketone body β-hydroxybutyrate also suppressed these abnormalities. Intact SIRT1 was necessary for each of these interventions (high-fat diet, NAD^+ precursors, or PARP inhibitors), which suggests that hyperactive PARP leads to decreased NAD^+, decreased SIRT1 activity, and defective mitochondrial function, thus resulting in an energy deficit and increased oxidative stress (Fig. 4). The ketone body β-hydroxybutyrate inhibits class I histone deacetylases (HDAC1, HDAC2, and HDAC3) and increases expression of MT2 and FOXO3A (*62*). Because FOXO proteins regulate NAMPT, oxidative stress, and aging, β-hydroxybutyrate may indirectly regulate NAD^+ concentrations, sirtuin function, and aging.

A similar model based on PARP activation and NAD^+ depletion was invoked for aging in mice and worms. NAD^+ concentrations are reduced in aged mice and worms, and restoring NAD with nicotinamide riboside or PARP inhibitors prevents age-associated metabolic decline and promotes longevity in worms. These effects depend on the SIRT1 worm ortholog, sir-2.1, and induction of mitonuclear protein imbalance (*48*).

Another parallel situation has been described in the case of cyclic ADP-ribose synthases (CD38, CD157) and brings further support for the model proposed above. Both proteins produce cyclic ADP-ribose from NAD^+ and were initially described as lymphocyte-specific ectoenzymes. However, they occur in other tissues, both inside and outside cells. CD38 is a major NAD^+-consuming enzyme, and mice lacking CD38 show increased NAD^+ concentrations in the brain, liver, and muscles. Furthermore, due to their resulting enhanced energy expenditure and higher metabolic rates, these animals are protected against obesity, despite a high-fat diet (*63*–*65*).

Their resistance to obesity is mediated in part through an NAD^+-dependent activation of the SIRT-PGC1α axis (*65*). In contrast, CD38 overexpression in a cell line that resulted in a 35% decrease of cellular NAD^+ concentrations was associated with a lower growth rate, increased oxidative stress, and decreased expression of proteins that function in glycolysis, antioxidant response, and DNA repair (*66*).

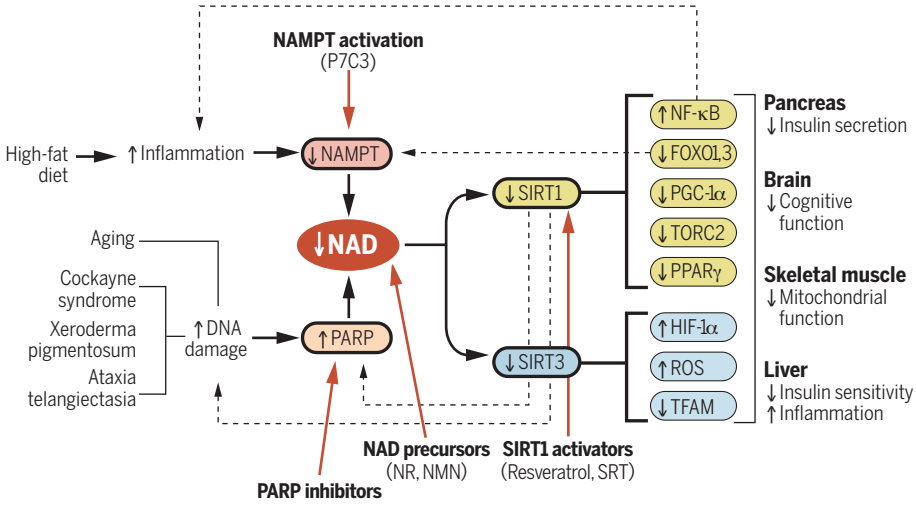

Fig. 4. A model for DNA damage, inflammation, NAD^+, and aging. Two key events, the activation of PARP by DNA damage and the decreased NAMPT expression associated with inflammation, lead to decreased SIRT1 and SIRT3 activity in the nucleus and mitochondria, respectively. Decreased SIRT1 activity is associated with further PARP activation and increased DNA damage. Decreased SIRT1 also leads to NF-κB activation and decreased FOXO3a activity, two factors that lead to increased inflammation. These contribute to the establishment of two parallel feed-forward self-reinforcing loops that further accelerate the aging process. This process is initiated earlier and faster in patients with DNA damage repair defects (such as CS, XPA, and AT). Mitochondrial function is diminished as a result of decreased SIRT3 activity, leading to mitochondrial protein hyperacetylation, whereas decreased SIRT1 is associated with decreased TFAM (necessary for mitochondrial DNA replication and transcription) and decreased PGC-1α (necessary for mitochondrial biogenesis). Possible therapeutic interventions to restore NAD^+ levels are illustrated for each of the key enzymes (red arrows).

Nutritional precursors for NAD^+ synthesis

As the field focuses on supplementing NAD precursors to remedy the decrease in NAD^+ that occurs with aging, our understanding of the mechanisms of precursor import and conversion is still rudimentary. Precursors for NAD^+ synthesis can be provided by the diet in several forms (Figs. 2 and 3). Tryptophan feeds into the de novo synthesis pathway and could be an important precursor for NAD^+, but the activity of ACMS decarboxylase diverts ACMS from NAD synthesis and may explain why tryptophan is a relatively poor NAD^+ precursor (*6*).

Intestinal brush border cells hydrolyze NAD^+ into NMN and 5′-AMP. NMN is hydrolyzed to NR and nicotinamide (*67*). Nicotinic acid can be derived directly from diet or the activity of nicotinamide deamidase in the gut microbial community. Inside the cell, nicotinic acid feeds into the Preiss-Handler pathway. NR can also be obtained from the diet (milk) and from partial digestion of NAD^+ and NMN. The fate of NMN and NR after ingestion as supplements or after injection is not clear. The cell-surface protein CD73 processes NAD^+ into NMN and NMN into NR, therefore providing a possible mechanism for entry of NMN and NAD^+ into cells (*68*, *69*) (Fig. 3).

NAD^+, mitochondrial function, and aging

Loss of mitochondrial function is a hallmark of aging and age-associated diseases (*70*). An interesting link has emerged between NAD^+ metabolism, SIRT1, SIRT3, and mitochondrial function. NR and PARP inhibitors increased life span in worms via activation of the mitochondrial unfolded protein response UPR^{mt} by sir2.1 (*48*). Because the ETC is a multiprotein complex made up of proteins encoded by both the nuclear and mitochondrial genomes, unbalanced gene expression from both genomes results in stoichiometric

variations between different subunits in complex I of the respiratory chain. These partially unfolded proteins activate the mitochondrial unfolded protein response UPR^{mt}. In a separate study, decreased nuclear NAD^+ was associated with defective SIRT1 activity and down-regulation of TFAM, the main transcription-replication factor for the mitochondrial genome. This resulted in an early decrease in the expression of mitochondrial genome–encoded proteins in the ETC and a pseudohypoxic state resembling the Warburg effect, with increased glycolysis and decreased activity of complexes I, III, and IV but not complex II, which is composed of only nuclear-encoded proteins (*71*). Short-term (1 week) supplementation of these mice with NMN restored mitochondrial homeostasis in muscles, which suggests that NAD^+ supplementation can restore some reversible aspects of the aging process (*71*). Both observations are consistent with the model indicating that an imbalance in the relative stoichiometries of mitochondria- versus nucleus-encoded ETC proteins may induce life-span extension via activation of the UPR^{mt}. In support of such a mechanism, mutation or reduced function in nuclear genes encoding ETC components in yeast, *Caenorhabditis elegans*, *Drosophila*, and mice increase life span through activation of the mitochondrial unfolded protein response UPR^{mt} (*72*).

Although SIRT1 has emerged as a key NAD^+ target in mediating its beneficial effects, mitochondrial NAD^+ metabolism and SIRT3 are also important in aging. SIRT3 is necessary for the protective effect of calorie restriction against age-associated hearing loss and oxidative stress (*30*, *34*). Furthermore, loss of SIRT3 is associated with induction of a pseudohypoxic state similar to that detected in aging (*71*). Loss of SIRT3 is associated with accelerated development of the metabolic syndrome in mice on a high-fat diet, and a SIRT3 polymorphism in humans is associated with decreased SIRT3 enzymatic activity and increased risk for the metabolic syndrome (*29*). Complex I is the major hydride acceptor from NADH in mitochondria, and inhibition of its activity traps the NADH in its reduced form (Fig. 3). Thus, loss of complex I of the respiratory chain through inactivation of one of its subunits (Ndufs4) is associated with an increase in NADH and a relative decrease in NAD^+. This decrease in NAD^+ leads to decreased SIRT3 activity, pronounced mitochondrial protein acetylation, and accelerated heart failure in response to chronic stress (*73*). Most of these consequences are rectified after supplementation with NMN (*73*). SIRT3 is also necessary for the protective effect of NR and Wld^S (a fusion protein encompassing NMNAT1, see below) against noise-induced hearing loss (*29*).

Defects of the mitochondrial ETC are one of the most frequent forms of human heritable metabolic diseases. Two recent studies tested NR supplementation in two mouse models of mitochondrial myopathy: Deletor mice carry a patient mutation in Twinkle, a mitochondrial replicative DNA helicase, and Sco2 knockout mice are characterized by impaired cytochrome c oxidase biogenesis (*74*, *75*). In both mouse lines, NR supplementation increased NAD^+ concentrations and mitochondrial biogenesis and also delayed disease progression (*74*, *75*), suggesting a protective role for NR against mitochondrial diseases and dysfunction associated with aging (*22*, *76*–*81*).

Variation in NAD^+ concentrations during aging

Nicotinamide adenine dinucleotide concentrations depend on several variables, including the cellular redox state (NAD^+/NADH ratio) and the rates of NAD^+ synthesis and NAD^+ consumption. NAD^+ is much more abundant than NADH, so we expect that variations in total NAD^+ concentration, rather than in the NAD^+/NADH ratio, are the main factor affecting sirtuin activity. However, changes in the NAD^+/NADH ratio have been reported in muscle during the aging process (*82*). Although classical experiments have suggested that NAD^+ concentrations were held constant (*83*), recent evidence indicates that cellular NAD^+ concentrations change under various conditions. Importantly, they fluctuate in a circadian manner, through activation of the NAD^+ salvage pathway (*22*, *80*).

Nicotinamide adenine dinucleotide concentrations increase in response to conditions associated with lower energy loads, such as fasting, glucose deprivation, calorie restriction, and exercise (*22*, *76*–*81*). In contrast, NAD^+ concentrations decrease in animals on high-fat diets (*46*, *84*–*88*) and during aging and senescence (*47*, *48*, *71*, *75*, *82*, *88*–*90*).

The fact that NAD^+ concentrations increase under conditions that increase life span or health span and decrease during aging or under conditions that decrease life span or health span supports the working model that decreased NAD^+ levels contribute to the aging process and that NAD^+ supplementation might exert protective effects during aging. Indeed, NAD^+ supplementation increases life span in yeast and worms (*48*, *91*).

Why do concentrations of NAD^+ decrease during the aging process (*47*, *48*, *71*, *75*, *82*, *88*–*90*)? The fact that supplementation with NMN (a product of NAMPT) corrects defects associated with aging may indicate that the NAD salvage pathway is deficient in aging. Decreased NAMPT expression occurs in several tissues (e.g., pancreas, white adipose tissue, and skeletal muscle) during aging (*89*), resulting from defective circadian rhythm regulation by CLOCK and BMAL (*22*) or from the oxidative stress and chronic inflammation associated with aging (*7*) (Fig. 4). NAD^+ might be consumed through hyperstimulation of NAD-consuming enzymes, particularly PARP1, the activity of which increases during aging as DNA damage accumulates (*47*, *48*).

Several key experiments highlight the protective role of NAD^+ and sirtuins in aging. First, NAD^+ and *SIR2* are critical for the life-span extension in response to calorie restriction in yeast (*9*). NAD^+ supplementation with NR increases life span in yeast and is dependent on NR kinases and SIR2 (*92*). NR also increases NAD^+ concentrations in mammalian cells and mice, activates SIRT1 and SIRT3, and causes increased oxidative metabolism and protection against metabolic abnormalities induced by consumption of a high-fat diet (*84*). NR promotes longevity in worms in a sir-2.1–dependent manner (*48*).

NAD^+ metabolism in obesity, fatty liver disease, and type 2 diabetes

Pancreatic β cells and neurons may represent distinct frailty points in NAD^+ metabolism: These tissues express the lowest amount of NAMPT, the rate-limiting enzyme in the NAD^+ salvage pathway (*7*). The roles of the two forms appear contradictory: Increased eNAMPT is linked with obesity, nonalcoholic fatty liver disease, and type 2 diabetes (*93*, *94*); in contrast, iNAMPT appears to decrease in abundance with age or in response to the inflammatory cytokine tumor necrosis factor α (*88*). Nutritional supplementation with the NAD^+ precursor NMN restores NAD^+ levels in mice with high-fat diet–induced type 2 diabetes and improves glucose intolerance and lipid profiles in older mice (*88*).

NAD^+ metabolism and neurodegeneration

Several key observations support a role for the NAD^+-sirtuin axis in neuroprotection. Axon degeneration has a key role in peripheral neuropathies; often precedes neuronal cell death in neurodegenerative disorders, such as Alzheimer's and Parkinson's disease; and can be induced directly by nerve injury in a process called Wallerian degeneration. A mouse strain called Wallerian degeneration slow mice (Wld^S) contains a spontaneous dominant mutation that delays this degeneration (*95*, *96*). In the Wld^S mouse, a 85-kb tandem triplication generates a fusion protein between Ufd2a (ubiquitin fusion degradation protein 2a) and the Nmnat1 protein, a key enzyme in the NAD^+ salvage pathway and the Preiss-Handler pathway (Fig. 2) (*97*). The Wld^S mutation protects against neuronal insults, including Parkinson's disease, hypoxic-ischemic injury, toxic neuropathy (taxol), and others (*97*). Although the native Nmnat1 is a predominantly nuclear protein, it is unclear whether the Wld^S fusion protein exerts its neuroprotective activity in the nucleus, axon, or mitochondria (*97*). The enzymatic activities of Nmnat1 and SIRT1 are necessary for the neuroprotective activity of Wld^S (*98*). Wld^S also protects against noise-induced hearing loss (*99*). Intense noise exposure causes hearing loss by inducing degeneration of spiral ganglia neurites that innervate cochlear hair cells. Administration of NR prevents noise-induced hearing loss and degeneration of spiral ganglia neurites, even after noise exposure. These effects appear to be mediated in the mitochondria, because SIRT3-overexpressing mice are resistant to noise-induced hearing loss and SIRT3 deletion abrogates the protective effects of NR and Wld^S in mice (*99*).

Further evidence for a role of NAD^+ metabolism in neuroprotection comes from a screen conducted in vivo to identify small molecules that enhance hippocampal neurogenesis in adult mice (*100*). An aminopropyle carbazole compound called P7C3 appeared to mitigate the death of newborn neurons. P7C3 was improved to yield derivatives

with marked neuroprotective activity in animal models of Parkinson's disease, amyotrophic lateral sclerosis, and concussive injury (*101*–*103*). This compound was identified as an allosteric activator of NAMPT, the rate-limiting factor in NAD^+ salvage, and was shown to protect cells treated with the NAD^+-depleting drug doxorubicin (*104*). Studies involving this compound have been limited to its neuroprotective effects, but aging rats treated with P7C3 maintained their body weight when compared to aged vehicle-treated rats (*104*). Thus, PC73 might influence other age-related conditions, such as frailty.

Conclusions

The body of work reviewed above indicates that decreased NAD^+ concentrations contribute to the aging process and to the pathogenesis of the chronic diseases of aging. These results also support the idea that NAD^+ supplementation protects against aging and its associated conditions. The experiments reviewed here also raise expectations that NAD^+ supplementation might be useful during normal human aging. Although such a prospect is clearly exciting, our understanding of NAD^+ metabolism and its regulation during aging in humans remain fragmentary. In particular, little is understood about the pharmacological properties of NR or NMN as drugs: How are NR and NMN modified by the gut microbiome? How are they absorbed? What is their fate in the blood and in different organs? What sirtuins are they activating and in which tissues? Most experiments with NR involved large amounts of this compound (400 mg/kg of body weight in mice, corresponding to 28 g for a 70-kg adult). This raises important concerns about the exact mode of action of NR or its absorption.

For clinical applications, it will be critical to conduct rigorous double-blind and placebo-controlled clinical trials in humans. More work will also be necessary to gain a full understanding of the role of sirtuin activation against aging. SIRT1 increases life span in mice only when overexpressed in a distinct region of the brain but not when overexpressed in the whole organism (*20*, *105*). Resveratrol and other sirtuin activators protect against the deleterious metabolic effects of the Western diet but initially did not appear to increase life span in mice (*106*–*109*). However, two recent studies show that SIRT1 activators can also modestly increase life span in mice on a regular chow diet (*110*, *111*).

Although SIRT1 activation is clearly protective in terms of metabolism, its increased activity might pose considerable risk in other organs. For example, SIRT1 is a key factor in T helper 17 CD4 cells that contribute to autoimmune disease when hyperactivated (*112*). Similarly, loss of SIRT1 or inhibition of its activity promotes the development of another CD4 T cell subset, the regulatory T cells, which protects against autoimmunity (*113*, *114*). Global SIRT1 activation, induced by SIRT1 activators or via an increase in NAD^+ concentration, might therefore place susceptible individuals at increased risk of autoimmune diseases. Similarly, because some tumors show increased NAMPT expression, interventions that increase NAD^+ might enhance or promote tumor development (*115*).

These concerns notwithstanding, the studies reviewed here have ignited considerable interest in manipulating NAD^+ concentrations in therapeutic efforts aimed at disease prevention and life-span extension. Future rigorous clinical testing in humans will tell us whether this early promise will become a reality.

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NAD^+ in aging, metabolism, and neurodegeneration

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