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NAD^+ metabolism in health and disease

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Nicotinamide adenine dinucleotide (NAD^+) is both a coenzyme for hydride-transfer enzymes and a substrate for NAD^+-consuming enzymes, which include ADP-ribose transferases, poly(ADP-ribose) polymerases, cADP-ribose synthases and sirtuins. Recent results establish protective roles for NAD^+ that might be applicable therapeutically to prevent neurodegenerative conditions and to fight *Candida glabrata* infection. In addition, the contribution that NAD^+ metabolism makes to lifespan extension in model systems indicates that therapies to boost NAD^+ might promote some of the beneficial effects of calorie restriction. Nicotinamide riboside, the recently discovered nucleoside precursor of NAD^+ in eukaryotic systems, might have advantages as a therapy to elevate NAD^+ without inhibiting sirtuins, which is associated with high-dose nicotinamide, or incurring the unpleasant side-effects of high-dose nicotinic acid.

The biology and biosynthesis of NAD^+

Nicotinamide adenine dinucleotide (NAD^+) and its phosphorylated and reduced forms, $NADP^+$, NADH and NADPH, have central roles in cellular metabolism and energy production as hydride-accepting and hydride-donating coenzymes. Discovery of the coenzymatic activity of NAD^+ is reviewed in Box 1 and the redox chemistry that is mediated by NAD^+ is schematized in Figure 1. Such reactions are not destructive in the sense that NAD^+ and NADH are interconverted by hydride transfer. As with all phosphorylated natural products, NAD^+ is biosynthesized from smaller units and is broken down. Whereas NAD^+ breakdown was thought once to be a nonspecific process, we now realize that NAD^+ consumption is linked intrinsically to signaling reactions inside and outside cells that control gene expression, Ca^{2+} mobilization, cell death and aging. In this review we provide a detailed overview of NAD^+ metabolism with emphasis on the potential for NAD^+-boosting therapies to maintain health and treat diseases.

Tryptophan is the *de novo* precursor of NAD^+ in all vertebrates and almost all eukaryotes investigated. People who subsist on tryptophan-poor diets run the risk of developing the nutritional deficiency pellagra unless their diet is supplemented with one of the classical vitamin precursors of NAD^+, nicotinic acid (Na) or nicotinamide (Nam), which are collectively termed niacin. As shown in Figure 2, the salvage pathways for the two niacins are encoded by different genes and, thus, might not be expressed equally in all vertebrate tissues. The blood–borne bacterium *Haemophilus influenza* can not synthesize NAD^+ *de novo* or salvage niacins (see Glossary). Instead, it depends on uptake of nicotinamide riboside (NR) and a bacterial pathway that converts NR to NAD^+ by phosphorylation and subsequent adenylylation [1]. Recently, it has become apparent that fungi and vertebrates encode eukaryotic NR kinases (Nrk isozymes) to salvage NR, a third vitamin precursor of NAD^+, which occurs in milk [2]. Precisely how and where, intracellularly and extracellularly, NR is produced is unknown and a matter of active research.

The abundance of NAD^+ in human cells is controlled by many factors. For example, genes such as *IDO*, which encodes the *de novo* biosynthetic enzyme indoleamine 2,3 dioxygenase, are under transcriptional control [3], whereas nicotinamide mononucleotide (NMN) adenylyltransferases (Nmnats), Nmnat1, Nmnat2 and Nmnat3, are localized differentially [4]. NAD^+ is partitioned into reduced (NADH), phosphorylated ($NADP^+$) and reduced, phosphorylated (NADPH) pools, in addition to the NAD^+ pool. Each pool resides differentially in membrane-bound compartments and is partially sequestered from free NAD^+ by binding to proteins.

Three classes of NAD^+ consumers

The abundance of NAD^+ is also regulated by breakdown, largely because the molecule is not only coenzyme for oxidoreductases but also a substrate for three classes of

Glossary

Ecto-enzymes: membrane-bound enzymes with an extracellular active-site.
Flushing: a painful condition that consists of 'hot flashes', reddening and heat in the extremities.
Reverse cholesterol transport: the multi-step process by which HDL particles deliver cholesterol to the liver for excretion through bile acids.
Salvage and *de novo* biosynthesis: biosynthetic pathways are termed salvage if the distinctive piece of the final product is recovered from breakdown products and *de novo* if the distinctive piece is produced from units that require large rearrangements. NAD^+ consists of an ADP-ribose group linked to Nam. Whereas the AMP moiety of ADP-ribose always derives from ATP, the distinctive piece of NAD^+ is nicotinamide, which is either salvaged or synthesized *de novo*. The three vertebrate salvage and one *de novo* NAD^+ biosynthetic pathways are depicted in Figure 2.

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* **Disclosure statement**

C.B. is inventor of intellectual property related to nicotinamide riboside kinases and uses of nicotinamide riboside. The intellectual property is owned by C.B.'s employer, Trustees of Dartmouth College, and therapeutic uses are licensed by Sirtris Pharmaceuticals, a firm for which C.B. serves on the Scientific Advisory Board.

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Box 1. History of the NAD$^+$ coenzyme

In the early 20th century, Arthur Harden and co-workers reconstituted cell-free glucose fermentation with two fractions, one termed 'zymase' that was heat-labile and retained by dialysis, and one termed 'cozymase' that was heat-stable and passed through dialysis. Zymase was not a purified enzyme but a protein fraction that contained glycolytic enzymes. The cozymase fraction contained ATP, Mg^{2+} and the NAD$^+$ coenzyme, the structure of which was determined by Otto Warburg. In glucose fermentation, NAD$^+$ functions as the hydride acceptor in the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase, producing NADH and diphosphoglycerate. Similarly, NADH functions as the hydride donor for alcohol dehydrogenase, which is required for the reduction of acetaldehyde to ethanol, regenerating NAD$^+$. Numerous hydride transfer enzymes or oxidoreductases interconvert either NAD$^+$ and NADH or NADP$^+$ and NADPH to reduce or oxidize small-molecule metabolites (see Figure 1 in the main text).

enzymes that cleave NAD$^+$ to produce Nam and an ADP-ribosyl product. Although, historically, these enzymes have been called NAD$^+$ glycohydrolases, NAD$^+$-dependent ADP-ribosyl transferase is a more precise term [5]. However, to avoid confusion with dedicated protein mono(ADP-ribosyl) transferases, we refer to the enzymes historically termed NAD$^+$ glycohydrolases as NAD$^+$ consumers. As depicted in Figure 3, the three classes of NAD$^+$ consumers are (i) ADP-ribose transferases or poly(ADP-ribose) polymerases, (ii) cADP-ribose synthases and (iii) sirtuins (type III protein lysine deacetylases).

The substantial flux through NAD$^+$-consuming pathways explains why people require niacin supplementation when tryptophan is limiting. If NAD$^+$ were only a coenzyme (i.e. not consumed but merely interconverted between oxidized and reduced forms by hydride transfer), the nutritional requirement to support ongoing synthesis in excess of that provided by the *de novo* pathway would be difficult to explain. Thus, we now believe that either dietary niacins or NR in conjunction with niacin and/or NR-salvage are required to maintain NAD$^+$ in cells that are undergoing rapid NAD$^+$ breakdown (Figure 2).

ARTs and PARPs

ADP-ribose transferases (ARTs) and the more numerous, poly(ADP-ribose) polymerases (PARPs) consume NAD$^+$ to create an ADP-ribosyl protein modification and/or to form the ADP-ribose polymer, PAR (Figure 3). In a comprehensive review of the function of ARTs and PARPs, de Murcia describes numerous conditions that induce this type of NAD$^+$ catabolism and the consequent ADP-ribose reaction products in DNA-damage responses, epigenetic modification, transcription, chromosome segregation and programmed cell death [6]. Because of the roles of PARPs in cell death, there are substantial pre-clinical and investigative efforts to inhibit PARP to protect against cardiac, inflammatory and neurodegenerative conditions [7]. However, because PARP has complex roles in cell survival and repair signaling in addition to mediating cell death, it might be difficult to develop neuroprotective strategies that involve chronic inhibition of this essential enzyme [7]. Moreover, much of the benefit associated with inhibition of PARP might be related to protecting cellular NAD$^+$, such that NAD$^+$-boosting therapies targeted to tissues in which PARP is activated might be safer and as effective.

cADP-ribose synthases

cADP-ribose synthases are a pair of ecto-enzymes also known as the lymphocyte antigens CD38 and CD157, which produce and hydrolyze the Ca^{2+}-mobilizing second-messenger cADP-ribose from NAD$^+$ [8–10] (Figure 3). CD38 catalyzes a base exchange between NADP$^+$ and Na to form Na adenine dinucleotide phosphate (NaADP) [11], which is also a hydrolytic substrate [12]. All the products of CD38 (cADP-ribose, ADP-ribose and NaADP) have distinctive roles in Ca^{2+} mobilization.

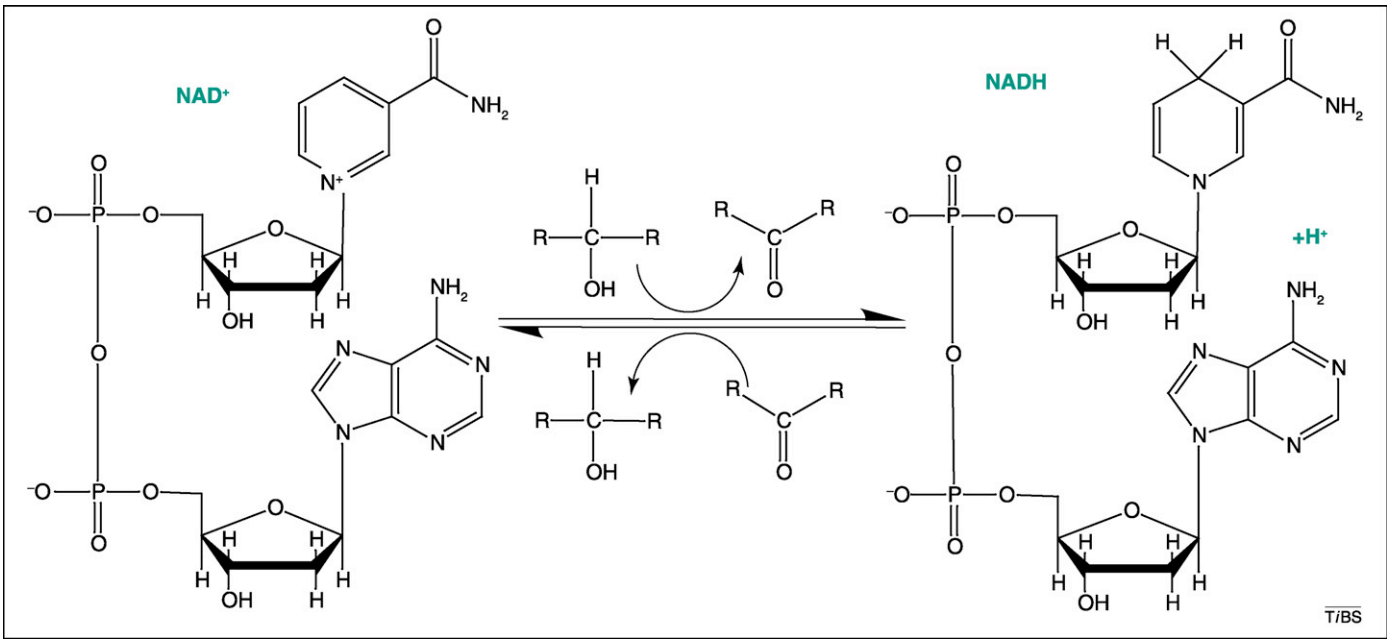

Figure 1. NAD$^+$ as a coenzyme for reversible hydride transfer. In a typical NAD$^+$-dependent oxidation, an alcohol is converted to the corresponding aldehyde with the production of NADH plus a proton. In the NADH-dependent direction, an aldehyde is reduced to an alcohol, which regenerates NAD$^+$.

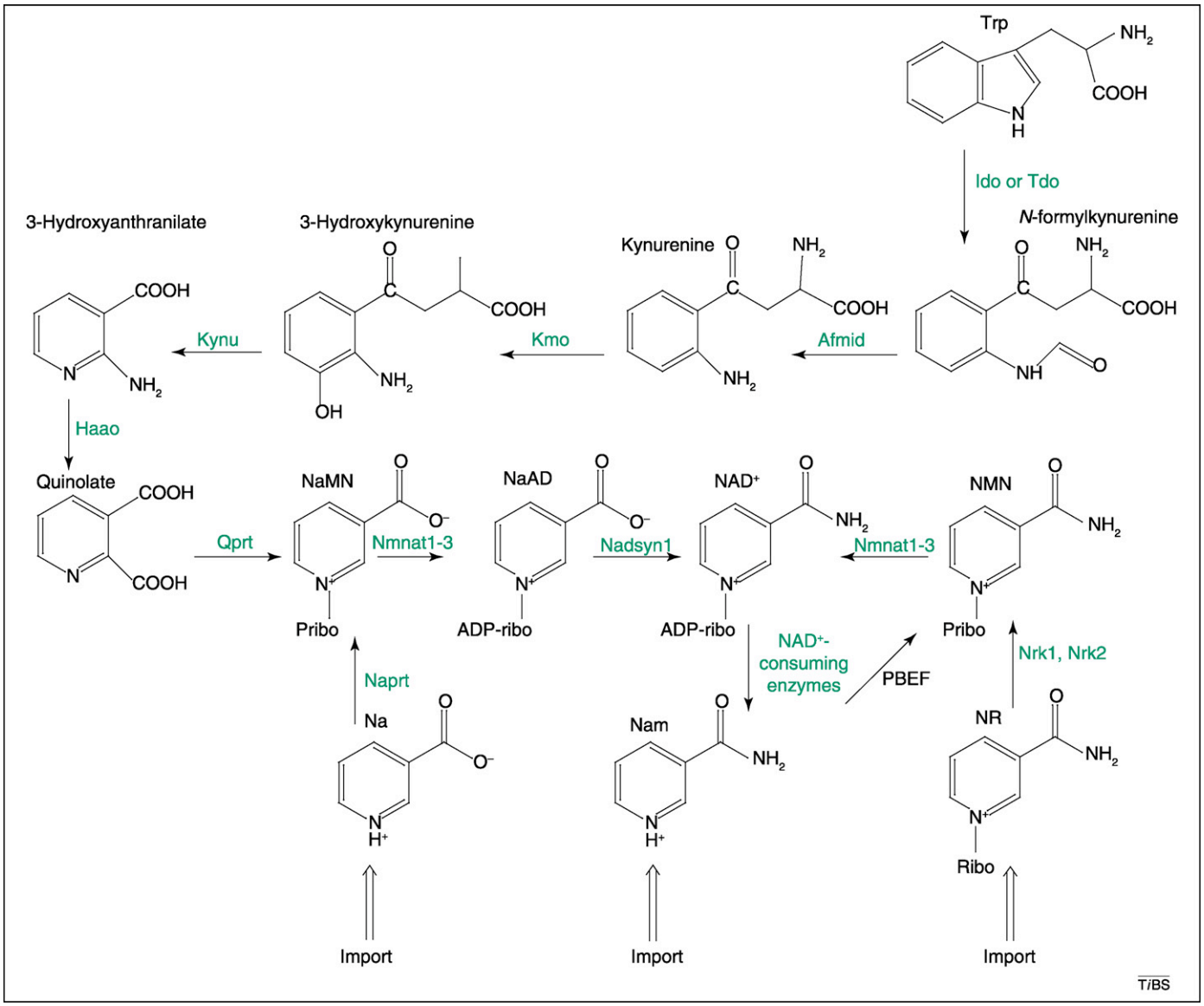

Figure 2. Intracellular NAD^+ metabolism in vertebrates. *De novo* synthesis begins with the conversion of tryptophan to *N*-formylkynurenine by either indoleamine dioxygenase (Ido) or tryptophan dioxygenase (Tdo). Arylformamidase (Afmid) then forms kynurenine, which is used as substrate by kynurenine monooxygenase (Kmo) to form 3-hydroxykynurenine. Kynureninase (Kynu) then forms 3-hydroxyanthranilate, which is converted to 2-amino-3-carboxymuconate semialdehyde (not shown) by 3-hydroxyanthranilate dioxygenase. The semialdehyde undergoes a spontaneous condensation and rearrangement to form quinolate, which is converted to NaMN by quinolate phosphoribosyltransferase (Qprt). NaMN is then adenylylated by Nmnat1, Nmnat2 and Nmnat3, to form nicotinic acid adenine dinucleotide ($NaAD^+$), which is converted to NAD^+ by glutamine-dependent NAD^+ synthetase (Nadsyn1). NAD^+-consuming enzymes (Figure 3) break the bond between the Nam and ADP-ribosyl moieties. Nam, which is also provided in the diet, is salvaged by a Nam phosphoribosyltransferase termed PBEF to NMN, which is adenylylated to form NAD^+ by Nmnat1, Nmnat2 and Nmnat3. Na, which is provided in the diet and, potentially, by bacterial degradative pathways in vertebrates, is salvaged by Na phosphoribosyltransferase (Naprt) to form NaMN. NR, which occurs extracellularly in blood and milk and can be provided in the diet, is salvaged by nicotinamide riboside kinases (Nrk1 and Nrk2). Na and Nam are also converted to nicotinuric acid and *N*-methylnicotinamide elimination products (not shown).

Sirtuins

Sirtuins, so named because of their similarity to yeast silent information regulator 2 (Sir2), are enzymes that function primarily in reversing acetyl modifications of lysine on histones and other proteins [5]. Also termed type-III histone deacetylases (HDACs), or, more precisely, type-III protein lysine deacetylases, sirtuins bind two substrates: the first is a protein or peptide that contains an acetylated lysine, and the second is NAD^+ [13] (Figure 3). Sirtuins position the leaving acetyl group to attack the ribose C1 carbon of the ADP-ribose moiety of NAD^+, which produces acetylated ADP-ribose plus Nam and the deacetylated protein lysine [14]. The acetylated ADP-ribose rearranges to form a mixture of 2′ and 3′ acetyl-ADP-ribose [15].

Sir2 was first identified as a positive regulator of gene silencing at cryptic mating-type loci. It functions in complexes that remodel chromatin to repress transcription and recombination in a manner that depends on reversing acetyl modifications on histone H3 and histone H4. Sirtuins from archaea, bacteria, yeast, invertebrates and vertebrates deacetylate histone and non-histone targets to alter enzyme activity and protein-complex formation, and to activate and repress transcription (Reviewed in [5]).

The relationships between sirtuins and ART activities are intimate and complex. For example, a trypanosomal sirtuin possesses both ART and deacetylase activity [16], whereas murine Sirt6 seems to be an ART but not a deacetylase [17]. In addition, activation of the Sir2 ortholog

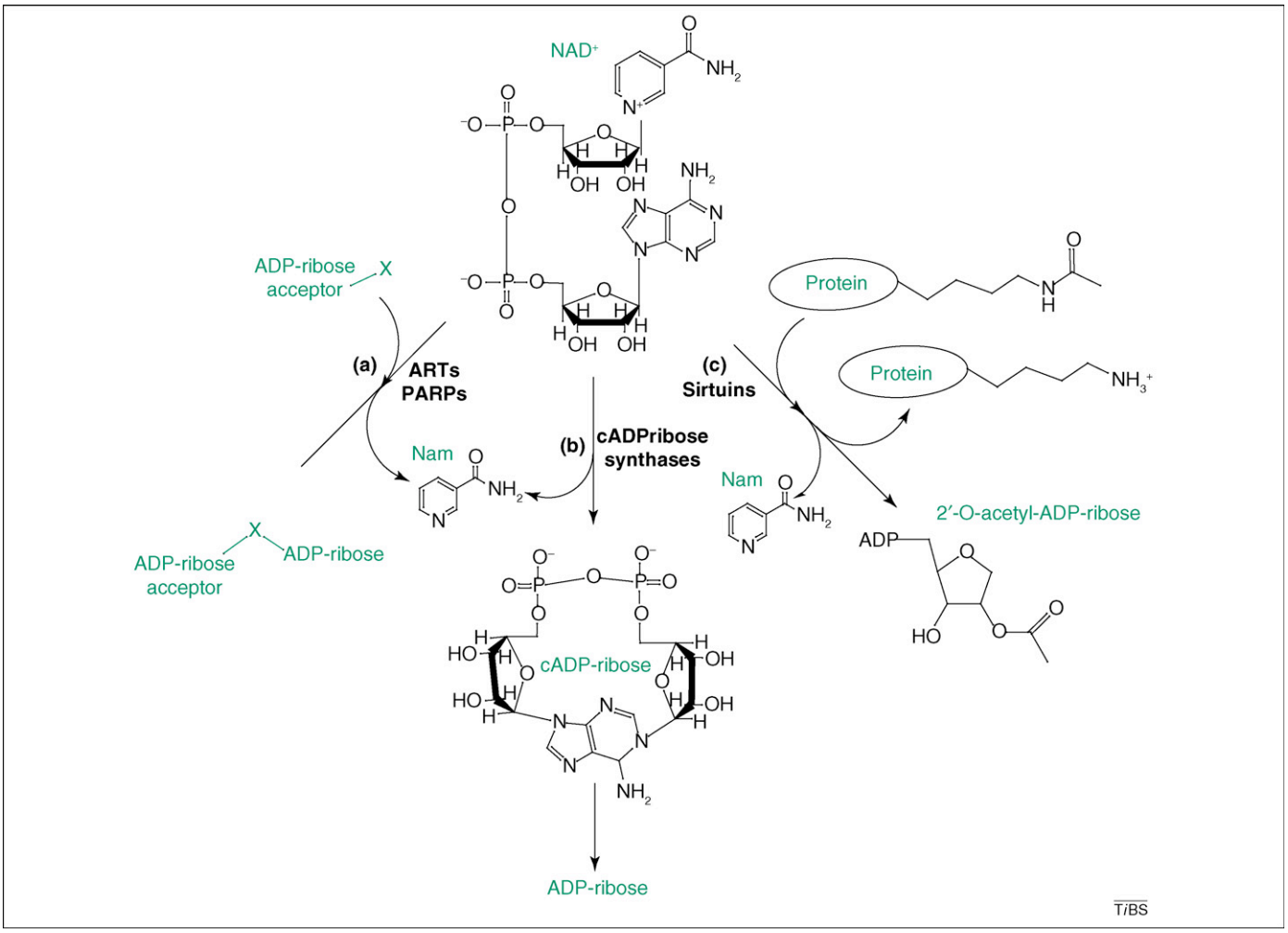

Figure 3. NAD^+ as a substrate for ADP-ribose transfer, cADP-ribose synthesis and protein lysine deacetylation. **(a)** ARTs and PARPs transfer ADP-ribose from NAD^+ as a protein modification with production of Nam. In the case of PARPs, the ADP-ribose acceptor, X, can also be ADP-ribose, forming poly(ADP-ribose). **(b)** cADP-ribose synthases cyclize the ADP-ribose moiety of NAD^+ with production of Nam. These enzymes also hydrolyze cADP-ribose. **(c)** Sirtuins use the ADP-ribose moiety of NAD^+ to accept the acetyl modification of a protein lysine, forming deacetylated protein plus Nam and, after acetyl-group rearrangement, a mixture of 2′ and 3′ O-acetylated ADP-ribose. Each NAD^+-consuming enzyme is inhibited by the Nam product.

Sirt1 is associated with reduction of PARP1 activity whereas deletion of Sirt1 increases the production of PAR [18]. Reciprocally, the cell death caused by activation of PARP1 in cardiac myocytes can be reduced by either administration of NAD^+ or increased NAD^+ biosynthesis, and the protective effect of NAD^+ biosynthesis depends largely on the presence of Sirt1 [19]. This constitutes direct evidence that therapy targeted to protect cellular NAD^+ is indicated by activation of PARP in heart failure.

Nam metabolism, and intracellular and extracellular NAD^+

A common theme among NAD^+ consumers is inhibition by Nam. ART, PARP [20], CD38 [21] and sirtuin [22] enzymes each contain a Nam-product site that can be occupied in the presence of substrates and enzyme intermediates. Thus, each enzyme can be inhibited by Nam, which effectively drives the formation of base-exchanged substrates. Because of this type of product inhibition, the salvage and/or elimination of Nam are crucial steps in NAD^+ metabolism.

Whereas Nam is salvaged to Na in fungi and many bacteria, the gene that encodes nicotinamidase is absent from vertebrate genomes and, thus, this route is not available to humans (except, potentially, in the gut where commensal bacteria might contribute to Nam salvage in the host). There is a human but not fungal gene encoding a Nam *N*-methyltransferase that converts Nam to *N*-methylnicotinamide *in vitro* [23]. In addition, humans express a homolog of the *H. influenza nadV* gene, which has Nam phosphoribosyltransferase activity [24] (Figure 2). Remarkably, this activity has been identified in a polypeptide named pre-B-cell colony-enhancing factor (PBEF), for which there are convincing reports of intracellular and extracellular localization. Intracellular PBEF increases NAD^+ concentrations and, as a consequence, has cell-protective benefits [19,24–27]. Extracellularly, PBEF has two known activities. First, the polypeptide synergizes with stem cell factor and interleukin 7 to promote the formation of pre-B-cell colonies [28]. Second, as an activity termed visfatin, PBEF is secreted by visceral fat, in order to bind the insulin receptor and mimic the effects of insulin [29]. The relationship between the enzymatic activity of PBEF and its extracellular activities has not been investigated. However, it is reasonable to suggest that PBEF functions, in part, by relieving Nam-mediated inhibition of an extracellular NAD^+-consuming enzyme

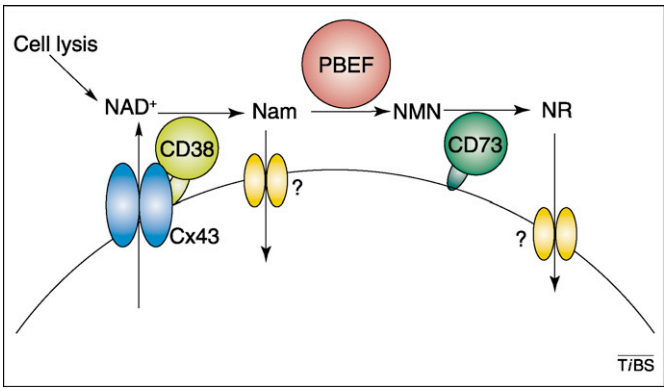

Figure 4. A potential extracellular NAD^+-cycle in vertebrates. Extracellular NAD^+ could be derived from ether cell lysis or, potentially, specific transport through Connexin 43 hemichannels. Extracellular cADP-ribose synthases, such as CD38, produce Nam from NAD^+. Nam might be converted to NMN by PBEF (Nam phosphoribosyltransferase) with subsequent dephosphorylation to NR by CD73. Nam and NR are thought to be imported by unidentified transporters.

and/or by participating in a NAD^+ biosynthetic cycle that is partially extracellular.

The source of NAD^+ for extracellular NAD^+ consumers such as ARTs and cADP-ribose synthase is a matter of interest. Although an obvious source of NAD^+ around sites of inflammation is cell lysis, there are strong indications that NAD^+ is transported via connexin 43 hemichannels, specifically to provide NAD^+ for the CD38 active site [30]. As shown in Figure 4, a potential extracellular NAD^+ biosynthetic cycle in vertebrates might be initiated by transport of NAD^+ by connexin 43 for consumption by CD38 to produce Nam and cADP-ribose. PBEF, with sufficient phosphoribosyl pyrophosphate (PRPP), might then convert Nam extracellularly to NMN [24]. In addition, CD73 – an ecto-enzyme that is homologous to nadN, the NMN nucleotidase of *H. influenza* [31] – might convert NMN to NR. Because *H. influenza* multiplies in vertebrate blood despite mutation of nadN [32], it is reasonable to surmise that NR circulates in vertebrate vascular systems and is taken up by cells that express a NR transporter.

NAD^+ synthesis in neuroprotection

Damage to nerve fibers leads to a series of molecular and cellular responses that are termed Wallerian degeneration or axonopathy. Axonopathy is a critical early event in distinct degenerative conditions including Alzheimer's disease (AD), Parkinson's disease and multiple sclerosis (MS), and it occurs in response to infections, alcoholism, acute chemotherapy-associated toxicity, diabetes and normal aging [33]. The Wallerian degeneration slow (wld^s) mouse is a spontaneous mutant that contains an autosomal dominant genetic alteration that confers resistance to nerve cell damage *ex vivo* and *in vivo*. The fusion protein encoded by wld^s contains the first 70 amino acids of ubiquitination factor Ufd2a and full-length Nmnat1, which is tandemly triplicated [34,35]. Overexpression of Nmnat1 blocks the axon degeneration induced by vincristine and transection in dorsal root ganglion (DRG) neurons [36,37]. It has also been shown that axonopathy is accompanied by depletion of NAD^+ and ATP, and that expression of wld^s protects neuronal NAD^+ levels [37].

Differences between the first two reports [36,37] of protection against axonopathy require reconciliation, especially in light of a putatively negative report [38] and subsequent clarifications of the protective value of NAD^+ synthesis in *ex vivo* [27] and *in vivo* [39] models of neurodegeneration. Milbrandt and co-workers have used lentiviral expression in DRG neurons to show that Nmnat1 protects against vincristine-induced axonopathy in an active-site dependent manner, and that bathing DRG neurons in 1 mM NAD^+ is also protective [36]. Based on RNAi-mediated knockdowns, they suggested that NAD^+-mediated protection depends on Sirt1 [36]. Independently, He and co-workers have corroborated protection by Nmnat1 and NAD^+, and provided evidence that exposure to vincristine and nerve transection lead to depletion of NAD^+. However, their study casts doubt on the role of Sirt1 as the key mediator of protection against axonopathy with the observation that embryonic *sirt1*−/− DRG neurons can be protected [37]. The report, which concludes that Nmnat1 does not substitute for the Wld^s fusion protein, actually confirms that lentiviral expression of Nmnat1 is protective, although possibly less so than full-length Wld^s [38]. This latter observation might be the result of a protein-stabilizing mechanism of the Ufd2a fragment. Although these investigators failed to produce a mouse transgenic for *nmnat1* that conferred a robust wld^s phenotype, their transgenes expressed Nmnat1 from the β-actin promoter [38] rather than the Ufd2a promoter, which is highly expressed in neurons [40].

Two more recent studies clarify the power, if not the precise mechanism, of NAD^+-mediated protection against neurodegeneration. The Milbrandt group developed lentiviral-expression systems for eight NAD^+ biosynthetic enzymes and examined the cellular localization and ability of these enzymes to protect mouse DRG axons from dying back after transection from neuronal cell bodies. Expression of Nam phosphoribosyltransferase and Na phosphoribosyltransferase genes protects, but only when neurons are cultured with Nam and Na, respectively, and neither Nam nor Na protects without overexpression of the corresponding biosynthetic gene. As in the earlier study, Nmnat1 protects without any remnant of Ufd2a but, in addition, a mutation that abolishes nuclear localization of Nmnat1 has no effect on protection, whereas expression of Nmnat3 either in the mitochondria (native) or nucleus (engineered) protects. These results indicate that neurons contain sufficient nicotinic acid mononucleotide (NaMN) or NMN for the increased expression of Nmnat to elevate NAD^+, and that increasing NAD^+ in the nucleus, cytoplasm or mitochondria protects against degeneration, which is ultimately a cytosolic process. Four compounds, NAD^+, NMN, NaMN and NR, protect without concomitant gene therapy. Although it is unclear how the non-drug-like phosphorylated compounds (NAD^+, NMN and NaMN) enter cells, and whether they are transported as nucleosides (NR and Na riboside), the data indicate that the Nrk pathway is primed to protect DRG neurons if the NR vitamin is supplemented. Indeed, the study shows that Nrk2 mRNA increases 20-fold in the 2 weeks following sciatic-nerve transection in rats [27].

A study in experimental autoimmune encephalomyelitis, a mouse model of MS, shows that the wld^s mutation has a

mild protective effect against development of neuromuscular deficits whereas Nam provides striking, dose-dependent delay and protection against the development of hind-limb weakness and paralysis. Indeed, Nam provides even greater protection to *wld*^{s} mice than to wild-type mice, probably because large doses of Nam render the Nmnat-biosynthetic step limiting [39]. In summary, the diseases and conditions that involve Wallerian degeneration (e.g. AD, chemotherapy-induced and diabetic-induced peripheral neuropathy, MS, and alcoholism) are collectively common and occur increasingly with advancing age. Thus, therapies that protect neuronal NAD^+ might prove to be quite powerful. However, the most effective compounds and formulations remain to be determined.

NAD^+ synthesis in candidiasis

Candida glabrata, the second leading cause of candidiasis, is a fungus with an interesting variation in NAD^+ metabolism. *Saccharomyces cerevisiae* NAD^+ metabolism differs from that of humans because the yeast lacks ARTs, PARPs and cADP-ribose synthases, and contains nicotinamidase (Pnc1) rather than PBEF. The *C. glabrata* genome is additionally missing genes for the *de novo* biosynthesis of NAD^+, such that it is a Na auxotroph [41]. Just as *S. cerevisiae* Sir2 represses transcription of sub-telomeric genes in an NAD^+-dependent manner [42], so *C. glabrata* Sir2 represses transcription of sub-telomeric *EPA1*, *EPA6* and *EPA7* genes, which encode adhesins that promote urinary-tract infection. Because *C. glabrata* cannot make NAD^+ *de novo*, low Na levels limit the function of Sir2, thereby derepressing adhesin genes and inducing a switch to adhere to host cells. As a consequence, increased dietary Na provides some protection against urinary tract infection in mice [41].

High-dose Na is a common, over-the-counter and prescription drug that increases high-density lipoprotein (HDL, otherwise known as 'good') cholesterol and reduces triglyceride levels [43] via an unknown mechanism. However, high-dose Na causes flushing via a receptor mechanism [44] that is unrelated to NAD^+ synthesis. Because patients with candidiasis might be particularly sensitive to flushing, we suggest that Nam and NR should be tested as anti-*C. glabrata* agents. The *C. glabrata* homologs of Pnc1 and Nrk1 are represented in National Center for Biotechnology Information (NCBI) databases (NCBI codes: CAG57733 and XP_448957, respectively). Nam might fail to support Sir2-dependent repression of adhesin genes because it is an inhibitor of Sir2 [45], which might result in adhesin gene derepression. Indeed, because there is no known route to produce Na in vertebrates, we suggest that *C. glabrata* is NAD^+-limited after depletion of NR, which is known to circulate based on the *Haemophilus* literature [32], and that the *C. glabrata* gene-expression switch might be either prevented or reversed by supplementation with NR.

NAD^+ synthesis in the regulation of aging

All fungi and animals that have been examined have characteristic rates of aging that depend on environmental conditions and yield mutations that confer either progeric or long-lived phenotypes. Calorie restriction (CR) is the most powerful intervention known to extend the lifespan of yeasts, worms, flies and mammals (Reviewed in [46]). CR increases lifespan and delays the onset of distinct debilitating diseases in different models. CR reduces carcinogenesis in mouse models, prevents kidney disease in rats, and forestalls diabetes and cardiovascular disease in monkeys. In addition, although there is no experimental proof that CR extends human lifespan, the hematological, hormonal and biochemical parameters of the eight people on a CR diet for almost two years in the Biosphere were similar to those of mice or monkeys on CR [47]. Although most humans would balk at CR diets that might leave us colder, smaller and lacking in sex drive, the longevity-promoting effects of CR are so profound in so many models that if we could understand the molecular basis of these effects, we might be able to develop tools to delay consequences of aging, and promote some of the cellular and physiological changes that occur in CR.

In yeast, the proximal cause of replicative senescence (failure of a mother cell to produce a daughter cell) of wild-type cells is the accumulation of extrachromosomal rDNA circles (ERCs) that are formed by recombination between tandemly arranged rRNA genes [48]. Thus, *sir2* mutants have shorter replicative life spans because Sir2 has a crucial role in repressing rDNA recombination [49].

In a landmark paper, Guarente and colleagues showed that CR extends replicative lifespan in yeast in a manner that depends on Sir2 and Npt1, the Na phosphoribosyltransferase [50]. The mechanisms by which CR promotes NAD^+-dependent activities of Sir2 might include increasing the NAD^+:NADH ratio [51], reducing inhibitory Nam [52], elevating NAD^+, and increasing levels of either Sir2 or specific Sir2–substrate complexes. Of the mechanisms that involve relief of inhibition, biochemical and cellular data suggest that Nam is a more effective inhibitor than NADH [22,53,54]. There are Sir2-independent mechanisms by which CR extends lifespan in yeast *fob1* mutants that do not accumulate ERCs [55]. The proximal causes of death in yeast *fob1* mutants and their interactions with CR are being pursued to identify additional targets that might be conserved in humans. There is also evidence that a Sir2-independent target of Nam limits the effects of CR [56], but much or all of this regulation might involve the paralogous sirtuin, Hst2 [57].

In worms and flies, increased gene dosage of the Sir2 ortholog extends lifespan [58,59] and, in flies, the beneficial effect of CR depends on dSir2 [59]. Hyperactivity, a physiological response to CR in vertebrates, depends on Sirt1 in mouse [60]. Because Sir2 has been conserved to alter gene expression in response to CR in metazoans, sirtuin activators are being developed as agents that might provide some of the benefits of CR. Resveratrol, a plant polyphenol that is enriched in red wine, was identified as an activator of human Sirt1 in a high-throughput screen [61]. Although activation by resveratrol depends on the identity of the Sirt1 substrate [62,63] and resveratrol has other targets in addition to Sirt1, multiple reports indicate that Sirt1 is one of the key targets of resveratrol [18,19,64]. Indeed, hard data now support the ability of high-dose resveratrol to increase the lifespan of worms and flies [65], and the health and vitality of overfed mice [66,67].

Although the data do not establish Sirt1 orthologs as the only mediators of the complex beneficial effects of resveratrol in vertebrates, increased mitochondrial biogenesis in liver [66] and muscle [67] can be explained by increased Sirt1-dependent deacetylation of the transcriptional coactivator, PGC-1α [68].

In the liver of mice fasted for 1 day, the levels of NAD^+ and Sirt1 are increased, which leads to Sirt1-dependent deacetylation of PGC-1α and consequent induction of gluconeogenic genes [68]. In a murine model of AD that overexpresses a human mutant amyloid β protein, CR increases levels of NAD^+ and Sirt1 and reduces Nam in the brain [69]. In this model, Sirt1 and NAD^+ reduce the production of amyloidogenic peptides and the resulting neuropathology [69]. Thus, although Sir2-dependent repression of the formation of ERCs is yeast-specific, there is broad conservation of CR, NAD^+ and sirtuin function. Moreover, the AD model indicates that specific NAD^+-boosting molecules might replace CR in treating specific diseases, which was a far from trivial assumption.

In cardiac and neuronal models, there are indications that some aspects of the protective effects of NAD^+ synthesis are mediated by sirtuins [19,69], but other effects might be sirtuin-independent [37]. In light of the localization of three human sirtuins to mitochondria, the control of mitochondrial acetyl-coA synthetase 2 by Sirt3 [70,71], and the connection between mitochondrial function and aging, a key area for future investigation to identify cell-protective and anti-aging targets of NAD^+ within mitochondria.

Na and plasma lipids

Finally, it is important to reinvestigate the mechanisms by which Na reduces levels of triglycerides and low-density lipoprotein cholesterol and elevates HDL cholesterol. It has long been assumed that the beneficial effects of Na on plasma lipids are mediated via a receptor rather than a vitamin mechanism because of the high dose required (100-fold higher than that required to prevent pellagra) and the failure of Nam to provide similar benefits [72]. Today, however, low HDL cholesterol and poor reverse cholesterol transport are regarded as a distinct molecular pathology and as risk factors for coronary heart disease [73] and AD [74]. Maintaining reverse cholesterol transport in the face of a distinct pathology might necessitate large doses of a vitamin, particularly because Na metabolism involves competition between synthesis and breakdown of NAD^+, production of nicotinuric acid, and metabolite excretion.

Although lack of a beneficial effect of Nam might be interpreted as evidence of a receptor-based mechanism, it is also consistent with a mechanism in which a sirtuin target is activated by NAD^+ and inhibited by high-dose Nam. Moreover, the Gpr109a receptor, which recognizes Na to the exclusion of Nam, mediates the flushing response [44], which is clearly an off-target effect. We suggest that in some individuals reverse cholesterol transport might be limited by a sirtuin-dependent deacetylation reaction such that high-dose Na and resveratrol both result in altered expression of apolipoproteins, transporters, receptors and/or enzymes that are involved in lipid metabolism. The ability to elevate NAD^+ with NR will enable the long-standing problem of the mechanism of action of NA in plasma-lipid homeostasis to be investigated.

Concluding remarks

The first century of NAD^+ research has been punctuated by multiple discoveries. Elucidation of the essential role of NAD^+ in glycolysis was followed by discoveries in human nutrition and coenzyme biosynthesis. In recent years, the role of NAD^+ in protein deacetylation has been discovered. NAD^+ precursors have been used to protect severed axons from degeneration, ameliorate neuromuscular deficits in a mouse model of MS and reduce the severity of candidiasis in a mouse model. Studies are needed to clarify the targets and mechanisms of NAD^+ function in these models, and to determine the safe, effective boundaries of nutritional and therapeutic interventions to replenish NAD^+ in humans.

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Erratum: NAD$^+$ metabolism in health and disease

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In the article 'NAD$^+$ metabolism in health and disease' by Peter Belenky, Katrina L. Bogan and Charles Brenner, which was published in the January 2007 issue of *Trends in Biochemical Sciences*, ribose moieties are incorrectly depicted as deoxyribose moieties in Figure 1. The correct figure is shown below. *Trends in Biochemical Sciences* apologises to the readers for the error.

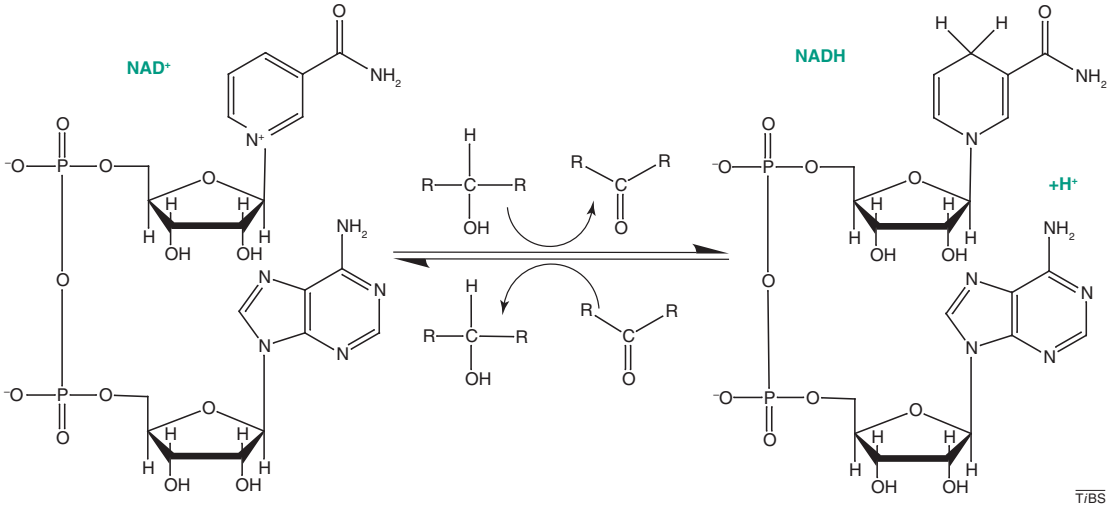

Figure 1. NAD$^+$ as a coenzyme for reversible hydride transfer. In a typical NAD$^+$-dependent oxidation, an alcohol is converted to the corresponding aldehyde with the production of NADH plus a proton. In the NADH-dependent direction, an aldehyde is reduced to an alcohol, which regenerates NAD$^+$.