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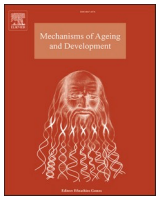

The balance between NAD^+ biosynthesis and consumption in ageing

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ABSTRACT

Nicotinamide adenine dinucleotide (NAD^+) is a vital coenzyme in redox reactions. NAD^+ is also important in cellular signalling as it is consumed by PARPs, SARM1, sirtuins and CD38. Cellular NAD^+ levels regulate several essential processes including DNA repair, immune cell function, senescence, and chromatin remodelling. Maintenance of these cellular processes is important for healthy ageing and lifespan. Interestingly, the levels of NAD^+ decline during ageing in several organisms, including humans. Declining NAD^+ levels have been linked to several age-related diseases including various metabolic diseases and cognitive decline. Decreasing tissue NAD^+ concentrations have been ascribed to an imbalance between biosynthesis and consumption of the dinucleotide, resulting from, for instance, reduced levels of the rate limiting enzyme NAMPT along with an increased activation state of the NAD^+-consuming enzymes PARPs and CD38. The progression of some age-related diseases can be halted or reversed by therapeutic augmentation of NAD^+ levels. NAD^+ metabolism has therefore emerged as a potential target to ameliorate age-related diseases. The present review explores how ageing affects NAD^+ metabolism and current approaches to reverse the age-dependent decline of NAD^+.

1. Introduction

NAD^+ is an essential molecule for all living organisms. NAD^+ and its phosphorylated form nicotinamide adenine dinucleotide phosphate ($NADP^+$) have a wide variety of functions, both in energy metabolism and in signalling pathways, and are involved in key cellular functions including replication, gene regulation and DNA repair (Nikiforov et al., 2015; Pollak et al., 2007). Originally, NAD and NADP were identified as redox coenzymes, with the capability to transfer electrons as part of the energy metabolism of the cell (Warburg and Griese, 1935). NAD-dependent electron transfer takes place in important metabolic pathways such as glycolysis and the Krebs cycle, making NAD a vital co-enzyme. NAD(P)-dependent electron transfer is carried out by a large group of enzymes known as dehydrogenases. These enzymes catalyse the reversible conversion between the oxidised forms of NAD(P), NAD^+ and $NADP^+$, and the reduced forms, NADH and NADPH, with the ratio between the two forms known as the redox state. Regulation of the redox state is important, not only for energy metabolism but also to provide cells with reducing species, such as NADPH, to prevent damage from free radicals (Agledal et al., 2010; Xiao et al., 2018; Ying, 2008). The redox state of $NADP^+$/NADPH in the cell is shifted towards NADPH, providing a readily available pool of reducing species (Tischler et al., 1977). The NAD^+/NADH ratio, on the other hand, is shifted towards NAD^+ (Lu et al., 2014; Zhang et al., 2002).

In addition to its role as an electron carrier, NAD^+ has been implicated as an important factor in a wide array of key cellular signalling pathways (Nikiforov et al., 2015; Berger et al., 2004; Houtkooper et al., 2010), and thus became subject of extensive research.

ADP-ribosylation is the transfer of an ADP-ribose (ADPR) moiety to a protein. This process was originally discovered in bacterial toxins (Moss and Vaughan, 1988). In these reactions, NAD^+ serves as a substrate for enzymes cleaving NAD^+ to add an ADPR moiety to an amino-acid acceptor. This transfer can be of a single ADPR moiety (mono-ADP ribosylation), or of multiple moieties by the formation of ADPR polymers (poly-ADP ribosylation) (Schreiber et al., 2006; Ame et al., 2004). In humans, mono- and poly-ADP ribosylation reactions are mainly catalysed by a family of enzymes referred to as the PARP family, based on the founding member poly ADPR polymerase 1, (PARP1). So far, 17 members of the PARP family have been identified, the majority of them mediating mono-ADP-ribosylation. Mono-ADP ribosylation has been found to play a role in the immune response, cell adhesion, the unfolded protein response and development of hippocampal neurons (Moss and Vaughan, 1988; Gupte et al., 2017). Poly-ADP ribosylation has important functions in a wide variety of cellular processes (D'Amours et al.,

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1999; Schiewer et al., 2012), most prominently in transcriptional regulation, DNA repair and initiation of cell death pathways (Schreiber et al., 2006; Wang et al., 2011).

Another function of NAD^+ is NAD-dependent deacylation, catalysed by sirtuins. The sirtuin protein family is homologous to the yeast silent information regulator 2 (ySir2), a protein found to increase life span in yeast by NAD-dependent deacetylation of telomere associated proteins (Denu, 2003). Homologues of the Sir2 protein are highly conserved in organisms ranging from archaea to humans, underlining their importance. Sirtuins deacylate their targets by cleaving NAD^+ and transferring the acyl group onto ADPR. Thus, in sirtuin-mediated protein deacetylation, the acetyl group is transferred from a lysine residue onto ADPR producing O-acetyl-ADP-ribose (OAADPR) and nicotinamide (Nam) (Jackson and Denu, 2002). In humans, the Sir2 homolog SIRT1 has been found to affect transcriptional activity by deacetylating and inactivating TAFI68, a TATA box binding protein (Muth et al., 2001). Another target for sirtuin deacetylation is the tumour suppressor gene p53, with deacetylation of p53 inducing repression of p53-dependent transcription and apoptosis (Langley et al., 2002; Vaziri et al., 2001). Notably, sirtuins have been the subject of extensive study largely because of the apparent link between sirtuin activity and regulation of life span by gene silencing (Blander and Guarente, 2004). The roles of sirtuins during aging will be discussed in greater detail below. OAADPR has been implicated in signalling processes such as gene silencing, ion channel gating and redox regulation (Tong and Denu, 2010). Nam has been shown to inhibit both PARPs and sirtuins, regulating these reactions by negative feedback (Uehara et al., 2006; Avalos et al., 2005).

NAD^+ also serves as a precursor for the calcium mobilizing signalling molecules generated by CD38/157 and SARM1. The conversion of NAD^+ to cyclic ADP-ribose (cADPR) occurs at neutral pH, while $NADP^+$ is converted to NAADP at acidic pH, through base exchange, where the Nam moiety is exchanged with nicotinic acid (NA) (Guse, 2015). These products then act as potent calcium-mobilizing agents. Additionally, NAD^+ is involved in the programmed axon degeneration pathway known as Wallerian degeneration, in which loss of activity of the NAD^+ biosynthetic enzyme Nicotinamide Mononucleotide Adenylyl Transferase 2 (NMNAT2) leads to accumulation of the NAD^+ precursor nicotinamide mononucleotide (NMN). This, in turn, activates the NAD^+ degrading enzyme SARM1, leading to NAD^+ depletion and degradation of the axon (Essuman et al., 2017; Conforti et al., 2014; Gerdts et al., 2016). As Nam is an important NAD^+ precursor as well as a product of NAD^+ consuming reactions, it provides a link between regulation of NAD biosynthesis and NAD^+ consuming reactions.

These non-redox functions of NAD^+ involve cleavage of the dinucleotide to donate ADPR or to generate second messenger molecules. As NAD^+ is constantly consumed in these reactions, there is a need for continuous NAD^+ biosynthesis. Imbalances between these NAD^+ consumption and NAD + biosynthesis may result in insufficient NAD^+ supply, which has been linked to several diseases, such as neuropathy, autoimmune diseases, and cancer. Given the importance of NAD^+ availability, it is interesting to note that NAD^+ levels decrease with ageing, and recently, a strong link has emerged between age-related NAD^+ decline and age-related diseases (Imai and Guarente, 2014; Verdin, 2015). In fact, enhancing NAD^+ levels by supplementation with NAD^+ precursors have been shown to be effective in increasing the life span of mice, yeast and *C. elegans* showing the therapeutic potential of modifying NAD^+ levels (Rajman et al., 2018; Yoshino et al., 2018; Mouchiroud et al., 2013; Fang et al., 2016; Belenky et al., 2007). Efforts to manipulate cellular NAD^+ levels mainly target the biosynthesis of NAD^+, and, in recent years, the NAD^+ biosynthetic pathways have been the subject of extensive study. In this review, we discuss how NAD^+ biosynthesis and NAD^+-dependent processes are affected by ageing and provide an outlook on how NAD^+ enhancing strategies can be utilised in reversing age-related NAD^+ decline.

2. NAD^+ biosynthesis

The constant degradation of NAD^+ by enzymes involved in signalling necessitates a continuous resupply of the dinucleotide, and the *in vivo* half-life of NAD^+ varies from 15 mins to 15 h depending on the tissue (Liu et al., 2018a). NAD^+ can be synthesised from dietary precursors or recycled from NAD^+ degradation products. Collectively the precursors, known as vitamin B3, are Nam, NA, nicotinamide riboside (NR), nicotinic acid riboside (NAR). In addition, the biosynthetic intermediate NMN can serve as precursor. Moreover, quinolinic acid (QA), a by-product of tryptophan catabolism, can be utilised to synthesise NAD^+ *de novo* (Bogan and Brenner, 2008; Magni et al., 2008; Bender, 1983). An overview of established mammalian NAD biosynthetic pathways is provided in Fig. 1. Very recently, dihydronicotinamide riboside (NRH) was identified as a novel NAD^+ precursor (Giroud-Gerbetant et al., 2019; Yang et al., 2020, 2019).

Most NAD^+ in mammals is generated in the salvage pathway, in which Nam, stemming from NAD^+-dependent signalling reactions, is resynthesised into NAD^+. Nam is converted to NMN by nicotinamide phosphoribosyltransferase (NAMPT), and the conversion of Nam to NMN is the rate limiting step in the salvage pathway. In the final step of NAD^+ biosynthesis via the salvage pathway, NMN is adenylated by NMNAT to generate NAD^+. NMN is also produced when NR is phosphorylated by nicotinamide riboside kinase (NRK) (Imai and Yoshino, 2013; Bieganowski and Brenner, 2004). In the Preiss-Handler pathway, nicotinic acid mononucleotide (NAMN) is synthesised from NA by nicotinic acid phosphoribosyltransferase (NAPRT). Subsequently, NAMN is converted to nicotinic acid adenine dinucleotide (NAAD) by NMNAT. In the final step NAAD is amidated by NAD synthetase (NADS) to NAD^+. NAMN is also generated by NRK when NAR is phosphorylated (Houtkooper et al., 2010; Preiss and Handler, 1958). *De novo* synthesis starts with the conversion of QA, a by-product of tryptophan catabolism, to NAMN by quinolinic acid phosphoribosyltransferase (QAPRT) which is further processed in the Preiss Handler pathway (Bender, 1983). Recently, NRH was discovered as a novel endogenous NAD precursor. NRH, which is present in the liver, is phosphorylated by adenosine kinase generating reduced NMN (NMNH) which in turn is adenylated to NADH by NMNAT (Yang et al., 2020).

Apart from NMNATs, all mammalian NAD biosynthetic enzymes localise to the nucleus/cytosol, and are encoded by single genes, except for the two NRK isozymes (Chiarugi et al., 2012). The three different NMNAT isozymes are encoded by different genes and differ in their oligomeric state, catalytic properties and subcellular localisation. NMNAT1 is found in the nucleus, NMNAT2 is bound to the cytosolic face of the Golgi apparatus (Schweiger et al., 2001; Berger et al., 2005; Lau et al., 2010; Mayer et al., 2010). At least one splice variant of *NMNAT3* encodes a mitochondrial matrix protein (Dolle et al., 2010). However, recent studies suggest NMNAT3 also to be localised to the mitochondrial intermembrane space or in endolysosomes (Berger et al., 2005; Davila et al., 2018; Nam et al., 2020).

The distribution of NAD^+ biosynthetic enzymes to different organelles allows for the establishment of separate organellar NAD^+ pools. The localisation of NMNAT1, 2, and 3 in the nucleus, cytoplasm and mitochondria, respectively, is of interest, since the majority of NAD^+-dependent metabolic pathways take place in these organelles (Liu et al., 2018a; Du et al., 2011; Schwer et al., 2002; Anderson et al., 2017). Establishing distinct pools may allow the cell to regulate NAD^+-dependent pathways within a compartment without affecting the whole cell by specifically altering the availability of the dinucleotide within an organelle. Differential regulation of *NMNAT1-3* expression, which all use NMN to synthesise NAD^+, may enable the cell to distribute NAD^+ to the organelle where it is most needed in a given situation. Recently, it was demonstrated that PARP1-dependent activation of adipocyte differentiation genes is regulated by restricting NMN and, in turn, NAD^+ from NMNAT1 in the nucleus by the upregulation of NMNAT2 expression in the cytoplasm (Ryu et al., 2018).

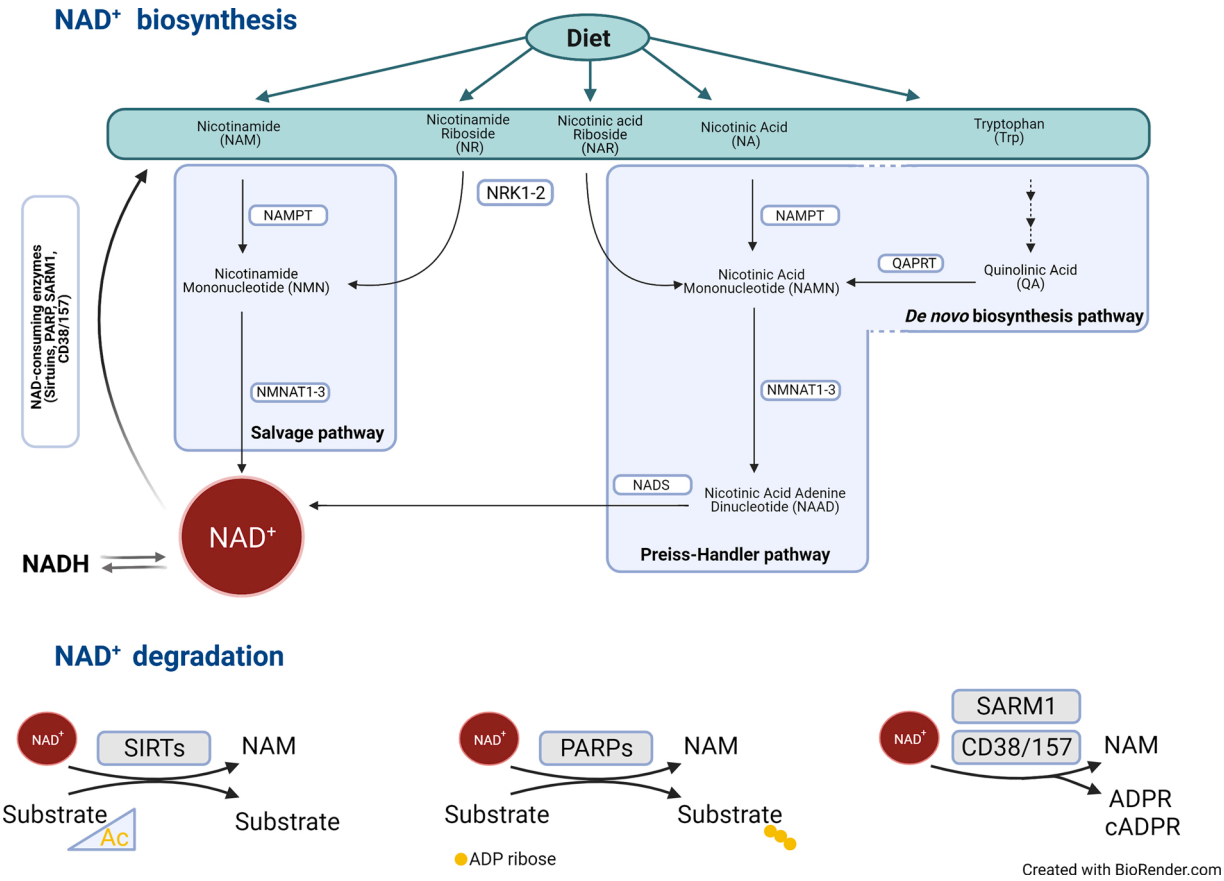

Fig. 1. NAD$^+$ biosynthesis and consumption. A) NAD$^+$ biosynthesis pathways start with various dietary precursors. NAD$^+$ can be synthesised from Nicotinic Acid (NA) in the Preiss-Handler pathway through three consequential enzymatic steps catalysed in order by NAPRT, NMNAT, and NADS. In the *de novo* pathway, also called the kynurenine pathway, NAD$^+$ is generated from quinolinic acid QA, a by-product of tryptophan catabolism, which is converted by QAPRT to NAMN. The salvage pathway starts with Nicotinamide (Nam), the major product of NAD$^+$ consuming enzymes, and involves two different enzymatic steps conducted by NAMPT and NMNATs. In addition, nicotinic acid riboside (NAR) and nicotinamide riboside (NR) are phosphorylated by NRK1/2 producing nicotinamide mononucleotide (NMN) which is further converted to NAD$^+$ B) NAD$^+$ consuming enzymes cleave NAD$^+$ into Nam and ADP-ribose (ADPR) or a ADPR derivative, in three different reaction types Sirtuins deacylates a broad range of substates, including acetylated proteins. Poly (ADP-ribose) polymerases (PARPs) attach a single ADPR moiety (mono-ADP ribosylation) or a polymer of ADPR moieties (poly-ADP ribosylation) onto a target, for example proteins or DNA. SARM1 and CD38/157, cleave NAD$^+$ into Nicotinamide, ADPR, and cADPR.

3. Impaired NAD$^+$ biosynthesis in ageing

Age-related NAD$^+$ decline has partially been attributed to a lower rate of NAD$^+$ biosynthesis. The majority of NAD$^+$ is generated in the salvage pathway and lowered expression levels of NAMPT during ageing have been identified as a major factor. The expression of NAMPT at both mRNA and protein levels is reduced in multiple tissues in aged mice (Yoshino et al., 2011; Stein and Imai, 2014). This age-related decline in NAMPT expression leads to a reduction of NAD$^+$ levels in the affected tissues, which in turn will affect the activities of NAD$^+$-dependent redox reactions and signalling processes. For example, the decline in proliferation and self-renewal of neural stem/progenitor cells during ageing has been tied to the reduced expression of NAMPT and the ensuing diminished NAD$^+$ levels (Stein and Imai, 2014).

The exact mechanism of age-related NAMPT decline is dependent on both the tissue and context, and several factors have been reported to affect NAMPT levels during ageing. Alterations of the circadian rhythm have been shown to contribute to age associated NAMPT decline. The core complex of transcription factors for the circadian rhythm, CLOCK: BMAL1, regulates *Nampt* expression by binding to the *Nampt* promoter region (Nakahata et al., 2009; Peek et al., 2013; Ramsey et al., 2009). SIRT1, an NAD$^+$ dependent deacetylase, regulates the expression of circadian genes by oscillatory deacetylation of BMAL1 and PER2 (Asher et al., 2008; Nakahata et al., 2008). Thereby, SIRT1 affects the stringency of circadian gene expression, and inhibition of SIRT1 reduces the circadian amplitude. This interdependency demonstrates that NAD$^+$, NAMPT and SIRT1 exist as a regulatory feedback loop that partially controls the circadian rhythm. Ageing affects both the rhythmic quality and amplitude of the circadian rhythm (Hood and Amir, 2017). This may reduce the expression of *Nampt* which in turn will lead to lower NAD$^+$ levels. In the suprachiasmatic nucleus of the hypothalamus of aged mice, a region of the brain that controls circadian rhythms *in vivo*, both NAMPT and SIRT1 are significantly reduced when compared to young mice (Chang and Guarente, 2013).

Another possible mechanism of age-related NAMPT decline is chronic inflammation. Cellular, oxidative and environmental stressors that promote chronic inflammation increase with ageing in different metabolic tissues including adipose, skeletal muscle and liver (Kourtis and Tavernarakis, 2011). In affected tissues, inflammatory cytokines, which exacerbate cellular damage, are released. These cytokines are reported to decrease the expression of *Nampt* (Yoshino et al., 2011; Kralisch et al., 2005). Interestingly, two of these cytokines, TNF-α and IL-1β, inhibit CLOCK:BMAL1-mediated gene expression (Cavadini et al., 2007; Petrzilka et al., 2009). As a result, chronic inflammation and the degradation of the circadian rhythm during ageing may synergistically reduce NAMPT levels.

MicroRNA, specifically mircoRNA-34a, contributes to hepatic NAD$^+$ decline during ageing. MircroRNA-34a binds to the 3' UTR region of

both *Nampt* and *Sirt1* and supresses their expression, which subsequently reduces the NAD^+ biosynthetic capacity of the affected tissue (Choi et al., 2013; Li et al., 2011; Yamakuchi et al., 2008). It has been demonstrated that the hepatic levels of mircroRNA-34a increase with age (Choi et al., 2013).

In comparison to our understanding of how ageing affects NAMPT, very little is known about how ageing impacts *de novo*- and Preiss Handler mediated NAD^+ biosynthesis. In particular, the role of NMNATs, which are the common enzymes for all the biosynthetic routes, remains elusive. In drosophila, which harbours only a single *Nmnat* gene, overexpression of NMNAT showed that oxidative stress biomarkers and ATP levels were improved. In addition, expression of longevity and mitochondrial related genes were increased (Liu et al., 2018b). This observation hints that NMNATs may affect the ageing process through their anti-oxidative stress and mitochondrial protection function. However, further research is needed to elucidate the role NMNATs may play in mammalian ageing.

4. NAD^+ signalling and degradation in ageing

The expression and activity of several NAD^+ consuming enzymes rise with age leading to increased consumption and lower levels of the dinucleotide. NAD^+ is consumed by sirtuins, PARPs, CD38/157 and SARM1, and these enzymes compete for NAD^+. Thereby, altering the activity or expression level of one enzyme affects the activity of the others (Fang et al., 2017).

In humans the sirtuin family is composed of seven members (SIRT1-SIRT7) which play a central role in several cellular metabolic processes related to health-span and longevity (Kanfi et al., 2012; Satoh et al., 2013). For instance, nuclear SIRT1, SIRT6 and SIRT7 are involved in DNA repair, while mitochondrial SIRT3, SIRT4 and SIRT5 and nuclear SIRT1 are involved in mitochondrial homeostasis (Carrico et al., 2018). Generally, sirtuins remove an acetyl group from lysine residues of target proteins. In addition, some sirtuins catalyse other reactions, such as, desuccinylation, demalonylation and fatty acid deacylation (Choudhary et al., 2014). SIRT1 plays a key role in mitochondrial biogenesis by regulating the peroxisome proliferator activated receptor-gamma co-activator-1alpha (PGC-1α) (Amat et al., 2009), as well as the turnover of defective mitochondria by mitophagy (Jang et al., 2012). Activation of the NAD^+/SIRT1-PGC-1α axis has therapeutic potential in neurodegenerative diseases, such as Huntington disease (Lloret and Beal, 2019), and amyotrophic lateral sclerosis (de la Rubia et al., 2019). The NAD^+/SIRT1-PGC-1α axis is also perturbed in accelerated ageing pathologies such as Ataxia telangiectasia, Cockayne syndrome and Xeroderma pigmentosum, which can partially be reversed by PARP1 inhibitors or supplementation with NAD^+ precursors (Fang et al., 2014; Scheibye-Knudsen et al., 2014). Tissues from aged mice show a decline in mitochondrial biogenesis associated with NAD^+ depletion and impaired SIRT1-PGC-1α signalling (Mouchiroud et al., 2013). Furthermore, SIRT1 and SIRT6 regulate the circadian rhythm (Masri et al., 2014).

PARP activity has been associated with age-related NAD^+ decline. Poly(ADP-ribosylation) is a key step in the recruitment and activation of several proteins involved in DNA repair, including single and double strand break repair (Ray Chaudhuri and Nussenzweig, 2017). Thus, PARPs are important in the early stages of DNA damage repair. PARP1, which accounts for 90 % of all PARP mediated DNA repair, is a major consumer of NAD^+ (Beck et al., 2014). Hyperactivation of PARP1 leads to cell death driven by mitochondrial apoptosis-inducing factor (AIF) (Wang et al., 2011), and ATP depletion through disrupted glycolysis (Andrabi et al., 2014). Notably, PARP1 inhibitors boost NAD^+ levels and increase SIRT1 activity and restore mitochondrial fitness and function (Pirinen et al., 2014). It has been suggested that increased PARP levels and activity are directly involved in the pathophysiology of age-related diseases, such as, Parkinson disease (PD) (Wu et al., 2014), ALS (Kim et al., 2004) and Alzheimer's disease (AD) (Kauppinen et al., 2011). PARP1 activation, NAD^+ decline and subsequent SIRT1 inhibition have been observed in patients with Progeroid disease, Ataxia telangiectasia, Xeroderma pigmentosum and Cockayne syndrome (Fang et al., 2014; Scheibye-Knudsen et al., 2014). This suggests PARP inhibitors can be a promising therapeutic target to ameliorate ageing-related diseases. For instance, the inhibition of PARP seems to prevent the pathologic α-synuclein toxicity, which is a key driver of neurodegeneration in PD (Kam et al., 2018). In addition, data suggest that the PARP-1/2 inhibitor, Veliparib, reduces the accumulation of cytoplasmic Tar DNA binding protein-43 (TDP-43), which plays a central role in ALS and frontotemporal dementia (McGurk et al., 2018). In addition, PARP1 inhibitors protect against the neuroinflammation and microglial activation associated with AD (Salech et al., 2020). PARP1 inhibition also promoted lifespan extension in mice with Cockayne Syndrome (Scheibye-Knudsen et al., 2014).

SARM1 is a modular protein that contains a catalytic Toll/interleukin-1 (IL-1) receptor (TIR) domain that hydrolyses NAD^+. Additionally, the enzyme can catalyse the formation of cADPR. SARM1 mediated NAD^+ depletion leads to Wallerian degeneration of damaged neurons, a key event in early stages of age-related neuronal disorders, such as PD, ALS, and AD (Salvadores et al., 2017). Accordingly, neural cells from PD patients exhibit increased SARM1 NAD^+ hydrolysis activity (Murata et al., 2018). Thus, targeting SARM1 emerges as a promising target to treat neuropathies. For example, data show that dominant-negative SARM1 adult mice are protected from early axon degeneration compared to wild type (Geisler et al., 2019). However, SARM1 gene knockout only prevents early axon degeneration but not long-term axonal loss (Wang et al., 2018; Viar et al., 2020). Therefore, further work is required to explore the protective role of targeting SARM1 enzymatic activity in ageing.

CD38 is a multifunctional protein that acts both as a receptor and an enzyme. As a receptor CD38, attaches to CD31 on the surface of T cells, stimulating cytokine production (Morandi et al., 2019). CD38 exists mainly as an ectoenzyme but can also be found bound to the plasma membrane facing the cytosol (Hogan et al., 2019). The enzyme is highly expressed throughout the immune system during inflammation, as well as in brain cells, such as neurons, astrocytes and microglia (Shubinsky and Schlesinger, 1997; Mizuguchi et al., 1995). In comparison CD157, a homolog of CD38, is expressed in endothelial cells and immune cells (Quarona et al., 2013). CD38 has emerged as one of the main drivers of NAD^+ depletion during ageing suggesting that targeting its NADase activity may restore NAD^+ levels. During ageing, CD38 expression increases in spleen, liver, and adipose tissue leading to lower NAD^+ levels and mitochondrial dysfunction (Camacho-Pereira et al., 2016). Increased expression of CD38 has been linked to inflammageing and neurodegenerative disease. Accordingly, cellular senescence, which is an age-related proliferation arrest, activates the expression of CD38 in tissue-resident macrophages (Covarrubias et al., 2020; Chini et al., 2020). Likewise, proinflammatory cytokines and chemokines, released from senescent cells, increase the activity and the expression level of CD38 (Chini et al., 2019). Furthermore, there is data suggesting that CD38 expression increases with AD disease progression in a double transgenic mouse model expressing a chimeric mouse/human amyloid precursor protein (APP) with the Swedish mutation (APP_{swe}) and a mutant presenilin 1 with the delta E9 ($PS1_{\Delta E9}$) compared to age-matched non-transgenic mice (Long et al., 2015). Similarly, an AD mouse model with CD38 knockout showed higher NAD^+ levels, and a milder disease phenotype with improved spatial learning (Blacher et al., 2015). Compared to CD38, little is known about the role CD157 may play in ageing. However, increased levels of CD157 have been related to inflammageing, autoimmune disease and certain types of cancer (Covarrubias et al., 2020; Ortolan et al., 2019).

Previous studies have provided compelling evidence on the role of NAD^+ consuming enzymes in ageing (Lautrup et al., 2019). However, further research is required to fully understand the underlying mechanisms of the age-associated activity changes of these enzymes, and the

interplay between NAD^+ depletion and ageing.

5. Therapeutic reversal of ageing-related NAD^+ decline

The importance of NAD^+ in longevity, ageing and health span is well established and the levels of the dinucleotide can be regulated by both lifestyle choices and dietary means. The age-related NAD^+ decline is due to an imbalance of NAD^+ synthesis and NAD^+ consumption capacities (Fig. 2). Several pharmacological approaches to address this imbalance have been established (Lautrup et al., 2019). To increase NAD^+ levels, dietary supplementation with NAD^+ precursors or activation of the rate limiting enzyme, NAMPT, has been explored (Gardell et al., 2019). *De novo* NAD^+ biosynthesis can be stimulated by inhibition of α-amino--β-carboxymuconate ε-semialdehyde decarboxylase (ACMSD) (Katsyuba et al., 2018). Alternatively, NAD^+ consumption can be lowered by inhibition of CD38 and PARPs (Boslett et al., 2017).

In yeast and *C. elegans* beneficial effects of NAD^+ precursors on the lifespan and healthspan have been firmly established (Mouchiroud et al., 2013; Fang et al., 2016; Belenky et al., 2007). Administration of low concentrations of Nam, μM range, to *C. elegans* increased lifespan (Mouchiroud et al., 2013). In contrast, higher doses of Nam, mM range, reduced lifespan (Schmeisser et al., 2013; Gallo et al., 2004). Thereby, the effect of Nam on lifespan extension is unclear. This discrepancy might be due to the inhibitory effect of Nam at high concentrations on sirtuins and PARPs (Avalos et al., 2005; Saldeen et al., 2003). Another possible explanation is that high concentrations of Nam can deplete cells of S-adenosyl methionine (SAM), a vital substrate for methylation reactions, as Nam is methylated to N-methyl Nam (MNAM) and secreted (Pissios, 2017). This process, in turn, might affect DNA methylation and lead to aberrant gene expression. MNAM has also been associated with cardiac disease, PD and type 2 diabetes (Hwang and Song, 2020). Additionally, the positive impact of NR supplementation on the lifespan of both *C. elegans* and yeast is well established. The effect in *C. elegans* has been reported to be dependent on sirtuin activity in many studies. In *C. elegans* models of various age-related disorders, Cockayne syndrome, ataxia telangiectasia, xeroderma pigmentosum group A and Werner syndrome, supplementation with NR or NMN extended lifespan (Fang et al., 2016, 2014; Scheibye-Knudsen et al., 2014; Fang et al., 2019).

In mice, most studies have been performed using different disease models and relatively little is known how NAD^+ precursor supplementation affects the longevity and healthspan of wild type mice. One study reported that long term administration of Nam has beneficial effects on the healthspan of mice fed a high fat diet. However, the lifespan was not affected (Mitchell et al., 2018). In another study supplementation with NR slightly increased the lifespan of old mice (Zhang et al., 2016). Long-term NMN supplementation, one year, starting at five months of age, enhanced physical activity, energy metabolism and insulin sensitivity with no signs of toxicity (Mills et al., 2016). In a mouse model of ataxia telangiectasia treatment with NMN increased NAD^+ levels and lifespan (Fang et al., 2016). In another study, using a mouse model of muscular dystrophy supplementation with NR enhanced muscle stem cell function by regulating mitochondrial metabolism (Zhang et al., 2016; Ryu et al., 2016).

The promising results in mouse models have prompted several clinical trials in humans, and an overview of the ongoing clinical trials can be found on clinicaltrials.gov. The safety, bioavailability and NAD^+ enhancing capacity of NR has been examined in two studies (Dellinger et al., 2018; Airhart et al., 2017). In one study the safety of supplementation with 250 or 500 mg NR daily for 60 days was assessed (Dellinger et al., 2018). In another study, participants were given 250 mg NR on day 1 and 2 and 1000 mg NR twice on day 7 and 8 and finally 1000 mg on day 9 (Airhart et al., 2017). In another trial, supplementation with 1000 mg NR for 21 days elevated the muscle NAD^+ metabolome, along with down-regulation of energy metabolism and mitochondrial pathways, while mitochondrial bioenergetics were unaffected. NR supplementation also reduced the levels of circulating

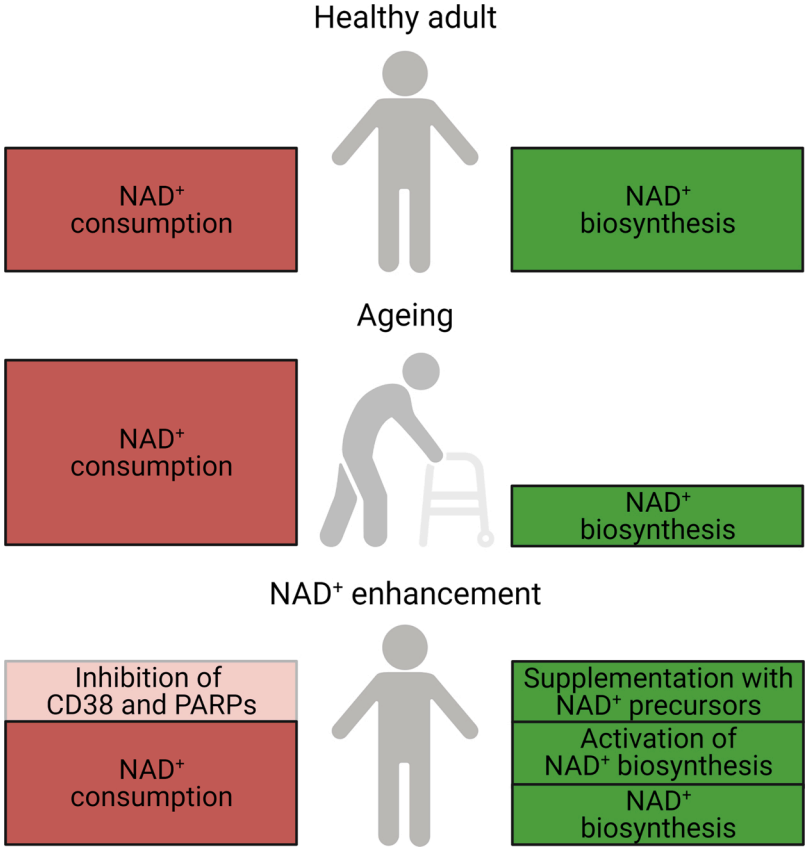

Fig. 2. Strategies for therapeutic reversal of age-dependent NAD^+ decline. NAD^+ levels are stable in healthy adults as the rate of biosynthesis and consumption is balanced. Ageing impairs NAD^+ biosynthesis as the levels of NAMPT decrease and increase consumption as the activity of PARPs and CD38 rise. Different therapeutic interventions to stop the age-dependent NAD^+ decline has been developed: The rate of NAD^+ consumption can be decreased by inhibition of CD38/157 and PARPs with small molecule inhibitors. NAD^+ biosynthesis can be increase by stimulation of the salvage pathway using small molecule activators of NAMPT. Alternatively, *De novo* synthesis of NAD^+ can be increased by inhibiting ACMSD. NAD^+ levels can be restored and increased by supplementation with NAD^+ precursors such as Nam, NR, NMN and NRH.

inflammatory cytokines (Elhassan et al., 2019). NR supplementation, 500 mg twice per day for 6 weeks, in healthy adults lowered blood pressure and reduced aortic stiffness but the results were not statistically significant. Other outcomes, such as physical activity, body fat, markers of exercise performance and glucose and insulin regulation did not improve (Martens et al., 2018). Another study explored the effect of NR supplementation, 1000 mg twice per day for 12 weeks, on insulin sensitivity and other metabolic parameters in obese, insulin-resistant men. The results showed that NR supplementation did not affect insulin sensitivity or glucose metabolism. No alterations in secondary outcomes were observed either (Dollerup et al., 2018). In these studies, the dosages and treatment regimens vary greatly, and future studies should focus on determining the best treatment regime and dosage. In comparison with NR, relatively little is known about the effect NMN supplementation has in humans, but several clinical trials are ongoing. The safety of NMN supplementation has been examined and doses up to 500 mg are considered safe in healthy males (Irie et al., 2020). In a recent study, NMN supplementation, 250 mg daily for 10 weeks, increased muscle insulin sensitivity and insulin signalling in prediabetic women who are overweight or obese (Yoshino et al., 2021).

Activation of NAD biosynthetic pathways also presents an intriguing way to counteract age-related NAD^+ decline. In particular, activation of NAMPT is of interest owing to its position as rate-limiting enzyme in the salvage pathway. Recently SBI-797812 was identified as a potent NAMPT activator which increased NMN production *in vitro*, in cells and *in vivo*, albeit only in the liver (Gardell et al., 2019). P7C3, a neuroprotective molecule, activates NAMPT and restores NAD^+ levels in doxorubicin treated cells, hinting the molecule may be an interesting therapeutic for age-related disorders (Wang et al., 2014). Activation of *de novo* NAD^+ biosynthesis by inhibition of ACMSD also increased the levels of the dinucleotide. The two ACMSD inhibitors TES-991 and TES-102524 increased NAD^+ levels and SIRT1 activity, improving mitochondrial function in the brain, liver and kidney of mice (Katsyuba et al., 2018). Future work should elucidate the exact molecular mechanisms of these activators and inhibitors and evaluate their suitability for the treatment of ageing-associated deficiencies in humans.

Inhibition of NAD^+ degrading enzymes has emerged as a powerful and promising approach to restore and increase levels of the dinucleotide during ageing. PARP1 inhibitors are commonly used as an adjunct to common cancer therapies as they make cells susceptible to DNA damage. However, PARP inhibition has also shown promise as an NAD^+ enhancer in ageing, and in *C. elegans* inhibition of PARPs significantly extends lifespan (Mouchiroud et al., 2013). Notably, several CD38 inhibitors have been developed or are being developed, because the enzyme is one of the main drivers of age-related NAD^+ decline (Camacho-Pereira et al., 2016). In mice, luteolin, a flavonoid, inhibits CD38, increases NAD^+ levels and protects the myocardium and endothelium following myocardial ischaemia (Boslett et al., 2017). In a clinical trial, luteolin had neuroprotective properties in children with autism (Taliou et al., 2013). Apigenin, another flavonoid inhibitor of CD38, increased NAD^+ levels in human cells and mouse tissues (Escande et al., 2013). It also improved lipid and glucose homeostasis in a mouse model of obesity (Escande et al., 2013). In a more recent study apigenin was found to not only inhibit CD38 but also lower the expression level of the enzyme. In the kidneys of diabetic rats apigenin increased the intracellular ratio of NAD^+/NADH and mitochondrial antioxidative enzyme activity was enhanced (Ogura et al., 2020). The compound 78c, a derivative of 4-aminoquinoline, inhibits CD38 and in mice increased the NAD^+ levels in heart, liver and muscle (Haffner et al., 2015). Treatment of mice with 78c prevents NAD^+ decline during ageing, and old mice treated with the compound exhibited improved muscle function, reduced DNA damage and ameliorated metabolic dysfunction (Tarrago et al., 2018). It has also been shown that 78c protects against postischemic endothelial and cardiac myocyte injury in mice (Boslett et al., 2019). DSRM-3716 was recently identified as potent inhibitor of SARM1 NADase activity. The compound phenocopied SARM1 depletion and protected axons from degeneration induced by axotomy or mitochondrial dysfunction. Additionally, DSRM-3716 rescued rotenone treated axons that already had entered the metastable state (Hughes et al., 2021). Before any of these inhibitors are used in clinical trials, their mechanism of action should be elucidated. In addition, it will be important to determine if they cause any deleterious side effects.

6. Conclusion and future perspectives

NAD^+ has in the past decade emerged as a key component in healthy ageing and longevity. Numerous excellent studies have demonstrated that there is an age-dependent reduction of NAD^+ levels. This decrease is partially due to an imbalance between NAD^+ biosynthesis and degradation, as the biosynthetic capacity is decreased, whereas NAD^+ degradation increases during ageing. Therapeutic augmentation of NAD^+ levels improves the health span and lifespan and alleviates the symptoms of various age-related pathologies in animal models. Supplementation with NR has shown some promise in clinical trials and numerous other trials are ongoing or planned. NMN supplementation clinical trials have only been initiated, but their results will be of great interest. Future work and clinical trials should also focus on activators and inhibitors of NAD^+ biosynthesis and NAD^+ degradation, respectively, as work in mice models have revealed encouraging effects.

Despite the considerable advances that have been made in the past decade, several questions remain unanswered. Perhaps, among the most pressing questions, the molecular mechanisms and pathways linking NAD^+ and ageing have so far remained elusive and not understood. Furthermore, the long-term effects of NMN and NR supplementation are not known, this is of importance since NAD^+ augmentation may also promote tumour growth in animal models.

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References

Agledal, L., Niere, M., Ziegler, M., 2010. The phosphate makes a difference: cellular functions of NADP. Redox Rep. 15 (1), 2–10.
Airhart, S.E., et al., 2017. An open-label, non-randomized study of the pharmacokinetics of the nutritional supplement nicotinamide riboside (NR) and its effects on blood NAD+ levels in healthy volunteers. PLoS One 12 (12) p. e0186459.
Amat, R., et al., 2009. SIRT1 controls the transcription of the peroxisome proliferator-activated receptor-gamma Co-activator-1alpha (PGC-1alpha) gene in skeletal muscle through the PGC-1alpha autoregulatory loop and interaction with MyoD. J. Biol. Chem. 284 (33), 21872–21880.
Ame, J.C., Spenlehauer, C., de Murcia, G., 2004. The PARP superfamily. Bioessays 26 (8), 882–893.
Anderson, K.A., et al., 2017. SIRT4 is a lysine deacylase that controls leucine metabolism and insulin secretion. Cell Metab. 25 (4), 838–855 e15.
Andrabi, S.A., et al., 2014. Poly(ADP-ribose) polymerase-dependent energy depletion occurs through inhibition of glycolysis. Proc. Natl. Acad. Sci. U. S. A. 111 (28), 10209–10214.
Asher, G., et al., 2008. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell 134 (2), 317–328.
Avalos, J.L., Bever, K.M., Wolberger, C., 2005. Mechanism of sirtuin inhibition by nicotinamide: altering the NAD(+) cosubstrate specificity of a Sir2 enzyme. Mol. Cell 17 (6), 855–868.
Beck, C., et al., 2014. Poly(ADP-ribose) polymerases in double-strand break repair: focus on PARP1, PARP2 and PARP3. Exp. Cell Res. 329 (1), 18–25.
Belenky, P., et al., 2007. Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD+. Cell 129 (3), 473–484.
Bender, D.A., 1983. Biochemistry of tryptophan in health and disease. Mol. Aspects Med. 6 (2), 101–197.
Berger, F., Ramirez-Hernandez, M.H., Ziegler, M., 2004. The new life of a centenarian: signalling functions of NAD(P). Trends Biochem. Sci. 29 (3), 111–118.
Berger, F., et al., 2005. Subcellular compartmentation and differential catalytic properties of the three human nicotinamide mononucleotide adenylyltransferase isoforms. J. Biol. Chem. 280 (43), 36334–36341.
Bieganowski, P., Brenner, C., 2004. Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD+ in fungi and humans. Cell 117 (4), 495–502.

Blacher, E., et al., 2015. Alzheimer's disease pathology is attenuated in a CD38-deficient mouse model. Ann. Neurol. 78 (1), 88–103.
Blander, G., Guarente, L., 2004. The Sir2 family of protein deacetylases. Annu. Rev. Biochem. 73, 417–435.
Bogan, K.L., Brenner, C., 2008. Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD+ precursor vitamins in human nutrition. Annu. Rev. Nutr. 28, 115–130.
Boslett, J., et al., 2017. Luteolinidin protects the postischemic heart through CD38 inhibition with preservation of NAD(P)(H). J. Pharmacol. Exp. Ther. 361 (1), 99–108.
Boslett, J., et al., 2019. Inhibition of CD38 with the thiazoloquin(az)olin(on)e 78c protects the heart against postischemic injury. J. Pharmacol. Exp. Ther. 369 (1), 55–64.
Camacho-Pereira, J., et al., 2016. CD38 dictates age-related NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism. Cell Metab. 23 (6), 1127–1139.
Carrico, C., et al., 2018. The mitochondrial acylome emerges: proteomics, regulation by sirtuins, and metabolic and disease implications. Cell Metab. 27 (3), 497–512.
Cavadini, G., et al., 2007. TNF-alpha suppresses the expression of clock genes by interfering with E-box-mediated transcription. Proc. Natl. Acad. Sci. U. S. A. 104 (31), 12843–12848.
Chang, H.C., Guarente, L., 2013. SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. Cell 153 (7), 1448–1460.
Chiarugi, A., et al., 2012. The NAD metabolome–a key determinant of cancer cell biology. Nat. Rev. Cancer 12 (11), 741–752.
Chini, C., et al., 2019. The NADase CD38 is induced by factors secreted from senescent cells providing a potential link between senescence and age-related cellular NAD(+) decline. Biochem. Biophys. Res. Commun. 513 (2), 486–493.
Chini, C.C.S., et al., 2020. CD38 ecto-enzyme in immune cells is induced during aging and regulates NAD(+) and NMN levels. Nat. Metab. 2 (11), 1284–1304.
Choi, S.E., et al., 2013. Elevated microRNA-34a in obesity reduces NAD+ levels and SIRT1 activity by directly targeting NAMPT. Aging Cell 12 (6), 1062–1072.
Choudhary, C., et al., 2014. The growing landscape of lysine acetylation links metabolism and cell signalling. Nat. Rev. Mol. Cell Biol. 15 (8), 536–550.
Conforti, L., Gilley, J., Coleman, M.P., 2014. Wallerian degeneration: an emerging axon death pathway linking injury and disease. Nat. Rev. Neurosci. 15 (6), 394–409.
Covarrubias, A.J., et al., 2020. Senescent cells promote tissue NAD(+) decline during ageing via the activation of CD38(+) macrophages. Nat. Metab. 2 (11), 1265–1283.
D'Amours, D., et al., 1999. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem. J. 342 (Pt 2), 249–268.
Davila, A., et al., 2018. Nicotinamide adenine dinucleotide is transported into mammalian mitochondria. Elife 7.
de la Rubia, J.E., et al., 2019. Efficacy and tolerability of EH301 for amyotrophic lateral sclerosis: a randomized, double-blind, placebo-controlled human pilot study. Amyotroph. Lateral Scler. Frontotemporal Degener. 20 (1–2), 115–122.
Dellinger, R.W., et al., 2018. Erratum: Author Correction: repeat dose NRPT (nicotinamide riboside and pterostilbene) increases NAD(+) levels in humans safely and sustainably: a randomized, double-blind, placebo-controlled study. NPJ Aging Mech. Dis. 4, 8.
Denu, J.M., 2003. Linking chromatin function with metabolic networks: Sir2 family of NAD(+)-dependent deacetylases. Trends Biochem. Sci. 28 (1), 41–48.
Dolle, C., et al., 2010. Visualization of subcellular NAD pools and intra-organellar protein localization by poly-ADP-ribose formation. Cell. Mol. Life Sci. 67 (3), 433–443.
Dollerup, O.L., et al., 2018. A randomized placebo-controlled clinical trial of nicotinamide riboside in obese men: safety, insulin-sensitivity, and lipid-mobilizing effects. Am. J. Clin. Nutr. 108 (2), 343–353.
Du, J., et al., 2011. Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. Science 334 (6057), 806–809.
Elhassan, Y.S., et al., 2019. Nicotinamide riboside augments the aged human skeletal muscle NAD(+) metabolome and induces transcriptomic and anti-inflammatory signatures. Cell Rep. 28 (7), 1717–1728 e6.
Escande, C., et al., 2013. Flavonoid apigenin is an inhibitor of the NAD+ ase CD38: implications for cellular NAD+ metabolism, protein acetylation, and treatment of metabolic syndrome. Diabetes 62 (4), 1084–1093.
Essuman, K., et al., 2017. The SARM1 toll/interleukin-1 receptor domain possesses intrinsic NAD(+) cleavage activity that promotes pathological axonal degeneration. Neuron 93 (6), 1334–1343 e5.
Fang, E.F., et al., 2014. Defective mitophagy in XPA via PARP-1 hyperactivation and NAD(+)/SIRT1 reduction. Cell 157 (4), 882–896.
Fang, E.F., et al., 2016. NAD(+) replenishment improves lifespan and healthspan in ataxia telangiectasia models via mitophagy and DNA repair. Cell Metab. 24 (4), 566–581.
Fang, E.F., et al., 2017. NAD(+) in aging: molecular mechanisms and translational implications. Trends Mol. Med. 23 (10), 899–916.
Fang, E.F., et al., 2019. NAD(+) augmentation restores mitophagy and limits accelerated aging in Werner syndrome. Nat. Commun. 10 (1), 5284.
Gallo, C.M., Smith Jr., D.L., Smith, J.S., 2004. Nicotinamide clearance by Pnc1 directly regulates Sir2-mediated silencing and longevity. Mol. Cell. Biol. 24 (3), 1301–1312.
Gardell, S.J., et al., 2019. Boosting NAD(+) with a small molecule that activates NAMPT. Nat. Commun. 10 (1), 3241.
Geisler, S., et al., 2019. Gene therapy targeting SARM1 blocks pathological axon degeneration in mice. J. Exp. Med. 216 (2), 294–303.
Gerdts, J., et al., 2016. Axon self-destruction: new links among SARM1, MAPKs, and NAD + metabolism. Neuron 89 (3), 449–460.
Giroud-Gerbetant, J., et al., 2019. A reduced form of nicotinamide riboside defines a new path for NAD(+) biosynthesis and acts as an orally bioavailable NAD(+) precursor. Mol. Metab. 30, 192–202.
Gupte, R., Liu, Z., Kraus, W.L., 2017. PARPs and ADP-ribosylation: recent advances linking molecular functions to biological outcomes. Genes Dev. 31 (2), 101–126.
Guse, A.H., 2015. Calcium mobilizing second messengers derived from NAD. Biochim. Biophys. Acta 1854 (9), 1132–1137.
Haffner, C.D., et al., 2015. Discovery, synthesis, and biological evaluation of thiazoloquin (az)olin(on)es as potent CD38 inhibitors. J. Med. Chem. 58 (8), 3548–3571.
Hogan, K.A., Chini, C.C.S., Chini, E.N., 2019. The multi-faceted ecto-enzyme CD38: roles in immunomodulation, cancer, aging, and metabolic diseases. Front. Immunol. 10, 1187.
Hood, S., Amir, S., 2017. The aging clock: circadian rhythms and later life. J. Clin. Invest. 127 (2), 437–446.
Houtkooper, R.H., et al., 2010. The secret life of NAD+: an old metabolite controlling new metabolic signaling pathways. Endocr. Rev. 31 (2), 194–223.
Hughes, R.O., et al., 2021. Small molecule SARM1 inhibitors recapitulate the SARM1(-/-) phenotype and allow recovery of a metastable pool of axons fated to degenerate. Cell Rep. 34 (1), p. 108588.
Hwang, E.S., Song, S.B., 2020. Possible adverse effects of high-dose nicotinamide: mechanisms and safety assessment. Biomolecules 10 (5).
Imai, S., Guarente, L., 2014. NAD+ and sirtuins in aging and disease. Trends Cell Biol. 24 (8), 464–471.
Imai, S., Yoshino, J., 2013. The importance of NAMPT/NAD/SIRT1 in the systemic regulation of metabolism and ageing. Diabetes Obes. Metab. 15 (Suppl. 3), 26–33.
Irie, J., et al., 2020. Effect of oral administration of nicotinamide mononucleotide on clinical parameters and nicotinamide metabolite levels in healthy Japanese men. Endocr. J. 67 (2), 153–160.
Jackson, M.D., Denu, J.M., 2002. Structural identification of 2'- and 3'-O-acetyl-ADP-ribose as novel metabolites derived from the Sir2 family of beta -NAD+-dependent histone/protein deacetylases. J. Biol. Chem. 277 (21), 18535–18544.
Jang, S.Y., Kang, H.T., Hwang, E.S., 2012. Nicotinamide-induced mitophagy: event mediated by high NAD+/NADH ratio and SIRT1 protein activation. J. Biol. Chem. 287 (23), 19304–19314.
Kam, T.I., et al., 2018. Poly(ADP-ribose) drives pathologic alpha-synuclein neurodegeneration in Parkinson's disease. Science 362 (6414).
Kanfi, Y., et al., 2012. The sirtuin SIRT6 regulates lifespan in male mice. Nature 483 (7388), 218–221.
Katsyuba, E., et al., 2018. De novo NAD(+) synthesis enhances mitochondrial function and improves health. Nature 563 (7731), 354–359.
Kauppinen, T.M., et al., 2011. Poly(ADP-ribose)polymerase-1 modulates microglial responses to amyloid beta. J. Neuroinflammation 8, 152.
Kim, S.H., et al., 2004. Widespread increased expression of the DNA repair enzyme PARP in brain in ALS. Neurology 62 (2), 319–322.
Kourtis, N., Tavernarakis, N., 2011. Cellular stress response pathways and ageing: intricate molecular relationships. EMBO J. 30 (13), 2520–2531.
Kralisch, S., et al., 2005. Interleukin-6 is a negative regulator of visfatin gene expression in 3T3-L1 adipocytes. Am. J. Physiol. Endocrinol. Metab. 289 (4), E586–90.
Langley, E., et al., 2002. Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. EMBO J. 21 (10), 2383–2396.
Lau, C., et al., 2010. Isoform-specific targeting and interaction domains in human nicotinamide mononucleotide adenylyltransferases. J. Biol. Chem. 285 (24), 18868–18876.
Lautrup, S., et al., 2019. NAD(+) in brain aging and neurodegenerative disorders. Cell Metab. 30 (4), 630–655.
Li, N., et al., 2011. Increased expression of miR-34a and miR-93 in rat liver during aging, and their impact on the expression of Mgst1 and Sirt1. Mech. Ageing Dev. 132 (3), 75–85.
Liu, L., et al., 2018a. Quantitative analysis of NAD synthesis-breakdown fluxes. Cell Metab. 27 (5), 1067–1080 e5.
Liu, X., et al., 2018b. Overexpression of Nmnat improves the adaption of health span in aging Drosophila. Exp. Gerontol. 108, 276–283.
Lloret, A., Beal, M.F., 2019. PGC-1alpha, sirtuins and PARPs in Huntington's disease and other neurodegenerative conditions: NAD+ to rule them all. Neurochem. Res. 44 (10), 2423–2434.
Long, A.N., et al., 2015. Effect of nicotinamide mononucleotide on brain mitochondrial respiratory deficits in an Alzheimer's disease-relevant murine model. BMC Neurol. 15, 19.
Lu, M., et al., 2014. Intracellular redox state revealed by in vivo (31) P MRS measurement of NAD(+) and NADH contents in brains. Magn. Reson. Med. 71 (6), 1959–1972.
Magni, G., et al., 2008. Enzymology of mammalian NAD metabolism in health and disease. Front. Biosci. 13, 6135–6154.
Martens, C.R., et al., 2018. Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD(+) in healthy middle-aged and older adults. Nat. Commun. 9 (1), 1286.
Masri, S., et al., 2014. Partitioning circadian transcription by SIRT6 leads to segregated control of cellular metabolism. Cell 158 (3), 659–672.
Mayer, P.R., et al., 2010. Expression, localization, and biochemical characterization of nicotinamide mononucleotide adenylyltransferase 2. J. Biol. Chem. 285 (51), 40387–40396.
McGurk, L., et al., 2018. Nuclear poly(ADP-ribose) activity is a therapeutic target in amyotrophic lateral sclerosis. Acta Neuropathol. Commun. 6 (1), 84.
Mills, K.F., et al., 2016. Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. Cell Metab. 24 (6), 795–806.

Mitchell, S.J., et al., 2018. Nicotinamide improves aspects of healthspan, but not lifespan, in mice. Cell Metab. 27 (3), 667–676 e4.
Mizuguchi, M., et al., 1995. Neuronal localization of CD38 antigen in the human brain. Brain Res. 697 (1–2), 235–240.
Morandi, F., et al., 2019. CD38, a receptor with multifunctional activities: from modulatory functions on regulatory cell subsets and extracellular vesicles, to a target for therapeutic strategies. Cells 8 (12).
Moss, J., Vaughan, M., 1988. ADP-ribosylation of guanyl nucleotide-binding regulatory proteins by bacterial toxins. Adv. Enzymol. Relat. Areas Mol. Biol. 61, 303–379.
Mouchiroud, L., et al., 2013. The NAD(+)/sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. Cell 154 (2), 430–441.
Murata, H., et al., 2018. c-Jun N-terminal kinase (JNK)-mediated phosphorylation of SARM1 regulates NAD(+) cleavage activity to inhibit mitochondrial respiration. J. Biol. Chem. 293 (49), 18933–18943.
Muth, V., et al., 2001. Acetylation of TAF(I)68, a subunit of TIF-IB/SL1, activates RNA polymerase I transcription. EMBO J. 20 (6), 1353–1362.
Nakahata, Y., et al., 2008. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell 134 (2), 329–340.
Nakahata, Y., et al., 2009. Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science 324 (5927), 654–657.
Nam, T.S., et al., 2020. Interleukin-8 drives CD38 to form NAADP from NADP(+) and NAAD in the endolysosomes to mobilize Ca(2+) and effect cell migration. FASEB J. 34 (9), 12565–12576.
Nikiforov, A., Kulikova, V., Ziegler, M., 2015. The human NAD metabolome: functions, metabolism and compartmentalization. Crit. Rev. Biochem. Mol. Biol. 50 (4), 284–297.
Ogura, Y., et al., 2020. CD38 inhibition by apigenin ameliorates mitochondrial oxidative stress through restoration of the intracellular NAD(+)/NADH ratio and Sirt3 activity in renal tubular cells in diabetic rats. Aging (Albany NY) 12 (12), 11325–11336.
Ortolan, E., et al., 2019. CD157: from immunoregulatory protein to potential therapeutic target. Immunol. Lett. 205, 59–64.
Peek, C.B., et al., 2013. Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. Science 342 (6158), p. 1243417.
Petrzilka, S., et al., 2009. Clock gene modulation by TNF-alpha depends on calcium and p38 MAP kinase signaling. J. Biol. Rhythms 24 (4), 283–294.
Pirinen, E., et al., 2014. Pharmacological Inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. Cell Metab. 19 (6), 1034–1041.
Pissios, P., 2017. Nicotinamide N-methyltransferase: more than a vitamin B3 clearance enzyme. Trends Endocrinol. Metab. 28 (5), 340–353.
Pollak, N., Dolle, C., Ziegler, M., 2007. The power to reduce: pyridine nucleotides–small molecules with a multitude of functions. Biochem. J. 402 (2), 205–218.
Preiss, J., Handler, P., 1958. Biosynthesis of diphosphopyridine nucleotide. I. Identification of intermediates. J. Biol. Chem. 233 (2), 488–492.
Quarona, V., et al., 2013. CD38 and CD157: a long journey from activation markers to multifunctional molecules. Cytometry B Clin. Cytom. 84 (4), 207–217.
Rajman, L., Chwalek, K., Sinclair, D.A., 2018. Therapeutic potential of NAD-boosting molecules: the in vivo evidence. Cell Metab. 27 (3), 529–547.
Ramsey, K.M., et al., 2009. Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. Science 324 (5927), 651–654.
Ray Chaudhuri, A., Nussenzweig, A., 2017. The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. Nat. Rev. Mol. Cell Biol. 18 (10), 610–621.
Ryu, D., et al., 2016. NAD+ repletion improves muscle function in muscular dystrophy and counters global PARylation. Sci. Transl. Med. 8 (361), p. 361ra139.
Ryu, K.W., et al., 2018. Metabolic regulation of transcription through compartmentalized NAD(+) biosynthesis. Science 360 (6389).
Saldeen, J., et al., 2003. Nicotinamide- and caspase-mediated inhibition of poly(ADP-ribose) polymerase are associated with p53-independent cell cycle (G2) arrest and apoptosis. Mol. Cell. Biochem. 243 (1–2), 113–122.
Salech, F., et al., 2020. Nicotinamide, a poly [ADP-ribose] polymerase 1 (PARP-1) inhibitor, as an adjunctive therapy for the treatment of Alzheimer's disease. Front. Aging Neurosci. 12, 255.
Salvadores, N., et al., 2017. Axonal degeneration during aging and its functional role in neurodegenerative disorders. Front. Neurosci. 11, 451.
Satoh, A., et al., 2013. Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. Cell Metab. 18 (3), 416–430.
Scheibye-Knudsen, M., et al., 2014. A high-fat diet and NAD(+) activate Sirt1 to rescue premature aging in cockayne syndrome. Cell Metab. 20 (5), 840–855.
Schiewer, M.J., et al., 2012. Dual roles of PARP-1 promote cancer growth and progression. Cancer Discov. 2 (12), 1134–1149.
Schmeisser, K., et al., 2013. Role of sirtuins in lifespan regulation is linked to methylation of nicotinamide. Nat. Chem. Biol. 9 (11), 693–700.
Schreiber, V., et al., 2006. Poly(ADP-ribose): novel functions for an old molecule. Nat. Rev. Mol. Cell Biol. 7 (7), 517–528.
Schweiger, M., et al., 2001. Characterization of recombinant human nicotinamide mononucleotide adenylyl transferase (NMNAT), a nuclear enzyme essential for NAD synthesis. FEBS Lett. 492 (1–2), 95–100.
Schwer, B., et al., 2002. The human silent information regulator (Sir)2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase. J. Cell Biol. 158 (4), 647–657.
Shubinsky, G., Schlesinger, M., 1997. The CD38 lymphocyte differentiation marker: new insight into its ectoenzymatic activity and its role as a signal transducer. Immunity 7 (3), 315–324.
Stein, L.R., Imai, S., 2014. Specific ablation of Nampt in adult neural stem cells recapitulates their functional defects during aging. EMBO J. 33 (12), 1321–1340.
Taliou, A., et al., 2013. An open-label pilot study of a formulation containing the anti-inflammatory flavonoid luteolin and its effects on behavior in children with autism spectrum disorders. Clin. Ther. 35 (5), 592–602.
Tarrago, M.G., et al., 2018. A potent and specific CD38 inhibitor ameliorates age-related metabolic dysfunction by reversing tissue NAD(+) decline. Cell Metab. 27 (5), 1081–1095 e10.
Tischler, M.E., et al., 1977. Pyridine nucleotide distributions and enzyme mass action ratios in hepatocytes from fed and starved rats. Arch. Biochem. Biophys. 184 (1), 222–236.
Tong, L., Denu, J.M., 2010. Function and metabolism of sirtuin metabolite O-acetyl-ADP-ribose. Biochim. Biophys. Acta 1804 (8), 1617–1625.
Uehara, N., et al., 2006. Nicotinamide blocks N-methyl-N-nitrosourea-induced photoreceptor cell apoptosis in rats through poly (ADP-ribose) polymerase activity and Jun N-terminal kinase/activator protein-1 pathway inhibition. Exp. Eye Res. 82 (3), 488–495.
Vaziri, H., et al., 2001. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell 107 (2), 149–159.
Verdin, E., 2015. NAD(+) in aging, metabolism, and neurodegeneration. Science 350 (6265), 1208–1213.
Viar, K., et al., 2020. Sarm1 knockout protects against early but not late axonal degeneration in experimental allergic encephalomyelitis. PLoS One 15 (6) p. e0235110.
Wang, Y., et al., 2011. Poly(ADP-ribose) (PAR) binding to apoptosis-inducing factor is critical for PAR polymerase-1-dependent cell death (parthanatos). Sci. Signal. 4 (167), ra20.
Wang, G., et al., 2014. P7C3 neuroprotective chemicals function by activating the rate-limiting enzyme in NAD salvage. Cell 158 (6), 1324–1334.
Wang, Q., et al., 2018. Sarm1/Myd88-5 regulates neuronal intrinsic immune response to traumatic axonal injuries. Cell Rep. 23 (3), 716–724.
Warburg, O.C.W., Griese, A., 1935. Hydrogen-transferring co-enzyme, its composition and mode of functioning. Biochemische Zeitschrift 280, 157–205.
Wu, X.L., et al., 2014. Effects of poly (ADP-ribose) polymerase inhibitor 3-aminobenzamide on blood-brain barrier and dopaminergic neurons of rats with lipopolysaccharide-induced Parkinson's disease. J. Mol. Neurosci. 53 (1), 1–9.
Xiao, W., et al., 2018. NAD(H) and NADP(H) redox couples and cellular energy metabolism. Antioxid. Redox Signal. 28 (3), 251–272.
Yamakuchi, M., Ferlito, M., Lowenstein, C.J., 2008. miR-34a repression of SIRT1 regulates apoptosis. Proc. Natl. Acad. Sci. U. S. A. 105 (36), 13421–13426.
Yang, Y., et al., 2019. Dihydronicotinamide riboside is a potent NAD(+) concentration enhancer in vitro and in vivo. J. Biol. Chem. 294 (23), 9295–9307.
Yang, Y., et al., 2020. NRH salvage and conversion to NAD(+) requires NRH kinase activity by adenosine kinase. Nat. Metab. 2 (4), 364–379.
Ying, W., 2008. NAD+/NADH and NADP+/NADPH in cellular functions and cell death: regulation and biological consequences. Antioxid. Redox Signal. 10 (2), 179–206.
Yoshino, J., et al., 2011. Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab. 14 (4), 528–536.
Yoshino, J., Baur, J.A., Imai, S.I., 2018. NAD(+) intermediates: the biology and therapeutic potential of NMN and NR. Cell Metab. 27 (3), 513–528.
Yoshino, M., et al., 2021. Nicotinamide mononucleotide increases muscle insulin sensitivity in prediabetic women. Science 372 (6547), 1224–1229.
Zhang, Q., Piston, D.W., Goodman, R.H., 2002. Regulation of corepressor function by nuclear NADH. Science 295 (5561), 1895–1897.
Zhang, H., et al., 2016. NAD(+) repletion improves mitochondrial and stem cell function and enhances life span in mice. Science 352 (6292), 1436–1443.