

Keeping the balance in NAD metabolism

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Abbreviations

ADPR, ADP-ribose; ARH1, ADP-ribosyl-acceptor hydrolase 1; ARTC, clostridial toxin-like ADP-ribosyltransferase; ARTD, diphtheria toxin-like ADP-ribosyltransferases; cADPR, cyclic ADP-ribose; Nam, Nicotinamide; NA, Nicotinic acid; NAAD, Nicotinic acid adenine dinucleotide; NAADP, Nicotinic acid adenine dinucleotide phosphate; NAD, Nicotinamide adenine dinucleotide; NADP, Nicotinamide adenine dinucleotide phosphate; NAMN, Nicotinic acid mononucleotide; NamPRT, Nicotinamide phosphoribosyltransferase; NAPRT, Nicotinic acid phosphoribosyltransferase; NAR, Nicotinic acid riboside; NMN, Nicotinamide mononucleotide; NMNAT, Nicotinamide mononucleotide adenyltransferase; NR, Nicotinamide riboside; NRK, Nicotinamide riboside kinase; PAR, Poly-ADP-ribose; PARG, Poly-ADP-ribose glycohydrolase; PARP, Poly-ADP-ribose polymerase; Pyruvate dehydrogenase complex (PDC) QA, Quinolinic acid; QAPRT, Quinolinic acid phosphoribosyltransferase; SARM1, Sterile alpha and Toll/interleukin-1 receptor motif containing 1; TARG1, Terminal ADP-Ribose Protein Glycohydrolase 1; Wld_s, Wallerian degeneration slow.

Abstract

Research over the last few decades has extended our understanding of nicotinamide adenine dinucleotide (NAD) from a vital redox carrier to an important signalling molecule that is involved in the regulation of a multitude of fundamental cellular processes. This includes DNA repair, cell cycle

regulation, gene expression and calcium signalling, in which NAD is a substrate for several families of regulatory proteins, such as sirtuins and ADP-ribosyltransferases. At the molecular level, NAD-dependent signalling events differ from hydride transfer by cleavage of the dinucleotide into an ADP-ribosyl moiety and nicotinamide. Therefore, non-redox functions of NAD require continuous biosynthesis of the dinucleotide. Maintenance of cellular NAD levels is mainly achieved by nicotinamide salvage, yet a variety of other precursors can be used to sustain cellular NAD levels via different biosynthetic routes.

Biosynthesis and consumption of NAD are compartmentalised at the subcellular level, and currently little is known about the generation and role of some of these subcellular NAD pools. Impaired biosynthesis or increased NAD consumption are deleterious and associated with ageing and several pathologies. Insults to neurons lead to depletion of axonal NAD and rapid degeneration, partial rescue can be achieved pharmacologically by administration of specific NAD precursors. Restoring NAD levels by stimulating biosynthesis or through supplementation with precursors also produces beneficial therapeutic effects in several disease models. In this review, we will briefly discuss the most recent achievements and the challenges ahead in this diverse research field.

Introduction

NAD is an important redox carrier mediating hydride transfer in oxido-reductive metabolic pathways, including the citric acid cycle, amino acid catabolism, β -oxidation of fatty acids and the urea cycle. In addition to its role as a redox carrier, NAD is an important signalling molecule involved in many vital cellular processes. For example, sirtuins are a class of NAD-dependent protein deacylases that display catalytic activity on a broad range of substrates (1) and regulate important processes such as cell cycle progression, circadian rhythm, genome stability, transcription, ageing, mitochondrial biogenesis and apoptosis (2,3). Sirtuin-mediated deacylation is coupled with cleavage of NAD into nicotinamide (Nam) and ADP-ribose (ADPR). The most common case is the deacetylation of proteins, where the leaving acetyl moiety is transferred onto ADPR, thereby forming O-acetyl-ADP-ribose (4) (Figure 1).

Of the NAD-dependent protein modifications, ADP-ribosylation was discovered first (5). In the reaction, one (mono-ADP-ribosylation) or several (poly-ADP-ribosylation) ADPR moieties from NAD are transferred onto acceptor proteins, releasing Nam (Figure 1). Two families of enzymes are responsible for ADP-ribosylation: diphtheria toxin-like ADP-ribosyltransferases (referred to as ARTDs or PARPs) and clostridial toxin-like ADP-ribosyltransferase (ARTCs) (6). ADP-ribosylation is tightly regulated and, like most other post-translational modifications, reversible. The cleavage of poly-ADP-ribose is largely performed by poly-ADP-ribose glycohydrolase (PARG), which cleaves the O-glycosidic bonds within polymers. However, PARG cannot remove the terminal ADPR moiety or reverse mono-

ADP-ribosylation, which instead is eliminated by enzymes such as ADP-ribosyl-acceptor hydrolase 1 (ARH1) and Terminal ADP-Ribose Protein Glycohydrolase 1 (TARG1) (7,8). ADP-ribosylation plays a role in innate immunity, DNA repair, transcription, cell cycle progression, energy metabolism, cell-cell interaction and a plethora of other processes (9,10).

In addition to its role in protein post-translational modifications NAD(P) is a precursor of nicotinic acid adenine dinucleotide phosphate (NAADP), cyclic ADP-ribose (cADPR) and ADPR, which are key regulators of calcium-signalling and synthesised by the multifunctional ADP-ribosyl cyclase CD38 and its homolog CD157 (Figure 1) (11,12). NAADP and cADPR stimulate release of calcium from endogenous stores, whereas ADPR triggers entry of calcium from the extracellular space (11,13-15). Maintenance of NAD-dependent signalling processes where NAD is cleaved hinges on a constant resupply of the dinucleotide.

Metabolism of NAD in humans

Under basal conditions, NAD-dependent protein deacetylation and ADP-ribosylation account for two thirds of net NAD consumption. The half-life of NAD *in vivo* varies between 15 min to 15 hours depending on the tissue, highlighting the importance of continuous re-synthesis of the dinucleotide (16). NAD is generated from dietary precursors or recycled from NAD degradation products. The precursors, collectively known as vitamin B3, are Nam and nicotinic acid (NA) as well as the nucleosides nicotinamide riboside (NR) and nicotinic acid riboside (NAR) (17,18). Additionally, quinolinic acid (QA), a product generated from tryptophan catabolism in the kynurenine pathway, is utilised to generate NAD *de novo* (Figure 1) (19).

In mammals, the most prominent pathway of NAD synthesis is the salvage pathway in which Nam, a by-product of both NAD dependent deacylation and ADP-ribosylation, is recycled to regenerate NAD. Nam is converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NamPRT). NMN is also produced when NR is phosphorylated by nicotinamide riboside kinase (NRK). In the final step of NAD biosynthesis, NMN is adenylylated by nicotinamide mononucleotide adenylyltransferase (NMNAT) to form NAD (20,21). In the Preiss-Handler pathway, nicotinic acid adenine dinucleotide (NAMN) is synthesised from NA by nicotinic acid phosphoribosyltransferase (NAPRT), NAMN is also generated when NAR is phosphorylated by NRK. The subsequent reaction catalysed by NMNAT yields nicotinic acid adenine dinucleotide (NAAD), which is then amidated to NAD by NAD synthetase (NADS) (22,23). *De novo* synthesis of NAD includes the conversion of QA to NAMN by quinolinic acid phosphoribosyltransferase (QAPRT) which is then further used in the Preiss-Handler pathway (Figure 1) (19). Recently it has been demonstrated that NR and NAR generated by dephosphorylation of the corresponding mononucleotides can be

secreted from cells, suggesting that different cell types can help maintain each other's NAD pools (24). NAD biosynthesis and NAD dependent signalling reactions are both compartmentalized in the eukaryotic cell, a fact that raises topological questions that remain unanswered.

Compartmentalisation of NAD biosynthesis and metabolism

NAD-dependent metabolic pathways predominate in the mitochondria and cytosol, which, along with the nucleus, are also the major compartments with non-redox functions of this dinucleotide. Basal cellular NAD consumption was reported to depend particularly on nuclear poly-ADP-ribosylation by PARPs/ARTDs 1/2 and on sirtuins 1/2-mediated protein deacetylation in the nucleus and the cytosol (16). Mitochondria contain three sirtuin isoforms (Figure 2), of which SIRT3 is the major protein deacetylase (25), whereas SIRT5 preferentially removes the acyl-groups of succinylated, malonylated and glutarylated proteins (26,27). SIRT4 is a bifunctional enzyme catalyzing both mono-ADP-ribosylation (28) and protein deacylation (29,30). As demonstrated for the mitochondrial Pyruvate dehydrogenase complex (PDC), the deacylation activity of SIRT4 is most efficient on lipoylated and biotinylated proteins (29,30).

A biosensor system based on immunodetection of poly-ADP-ribose (PAR) as readout allowed for indirect visualisation of NAD also in peroxisomes, the endoplasmic reticulum and the Golgi apparatus (31). However, the role and importance of these organellar NAD pools remain poorly characterised. Since biological membranes are impermeable for pyridine nucleotides, the presence of NAD in various subcellular compartments raises the question of how these individual pools are generated and maintained, and whether they are segregated or exchangeable.

The human genome harbours three genes encoding NMNAT isozymes, that differ in catalytic properties, oligomerisation state and subcellular localisation, namely, to the nucleus (NMNAT1) (32,33), the cytosolic face of the Golgi (NMNAT2) (34,35) and the mitochondria (NMNAT3) (Figure 2) (33). This distinct subcellular distribution overlaps with the localisation of major NAD consuming pathways. However, it differs from the nuclear-cytosolic localisation common to all other NAD biosynthetic enzymes (36). Moreover, with the exception of the two NRK isozymes, all other enzymes of NAD biosynthesis are encoded by single genes.

The subcellular location of NMNAT3 intuitively suggests that this isozyme is responsible for the synthesis of NAD in mitochondria, yet a mechanism for the supply of the substrate NMN into this organelle is elusive. The apparent absence of a pathway for autonomous NMN biosynthesis in mitochondria (37) was suggested to be bypassed by import of the mononucleotide from the cytosol (36), but a mitochondrial carrier for NMN awaits identification. Several recent studies challenge a

vital role of NMNAT3. For example, *Nmnat3*^{-/-} mice are viable and the absence of an NMNAT3 appears to affect metabolic functions rather in the cytosol than in mitochondria (38). NAD levels in skeletal muscle, which are elevated in transgenic *Nmnat3* mice (39), are hardly affected in *Nmnat3*^{-/-} mice (40). These findings suggest alternative mechanisms to compensate for the lack of NMNAT3. A mitochondrial carrier for NAD has been identified in yeast (41) and plants (42), and studies performed using isotopically labelled precursors (37) and a genetically encoded fluorescent NAD biosensor (43) provided supporting evidence for mitochondrial import of NAD, which may be generated by NMNAT2 in the cytosol (Figure 2). In humans, direct import of NAD through a biological membrane has so far only been suggested for peroxisomes. SLC25A17, a member of the solute carrier family of membrane transport proteins was shown to be able to carry NAD into reconstituted liposomes, indicating that it is a peroxisomal NAD carrier (44). The identity of a human mitochondrial NAD transporter remains unknown.

Embryonic lethality of both *NMNAT1*^{-/-} (45) and *NMNAT2*^{-/-} (46) mice implies important non-redundant roles for these two isozymes in the nucleus and the cytosol, and recent reports challenge the hypothesis that the nuclear membrane does not provide a diffusion barrier for pyridine nucleotides. For instance, studies performed with a fluorescent NAD biosensor indicated that the selective downregulation of cytosolic NMNAT2 does not affect the nuclear NAD pool (43). Using the same experimental tool, Ryu et al. suggested a distinct segregation of these two NAD pools related to a biological function. They demonstrated that the PARP1-dependent activation of genes involved in adipocyte differentiation is regulated by restricting NMN availability for nuclear NAD biosynthesis by NMNAT1 through stimulation of cytosolic NMNAT2 expression (47).

The role of NAD metabolism in maintaining axonal integrity

NAD metabolism has emerged as a key regulator of axonal degeneration. In injured axons, ATP loss and morphological degeneration are preceded by rapid NAD depletion (48,49). The seminal discovery of the mouse mutant Wallerian degeneration slow (*Wld_s*), whose peripheral nerves remain intact for 14 days following axotomy (Figure 3), led to the realisation that axonal degeneration is a regulated process. The underlying mutation for the expression of the *Wld_s* protein in these mice consists of a 85 kb- tandem triplication on chromosome 4 which leads to the generation of a chimeric protein composed of the 70 N-terminal residues from the ubiquitin ligase Ufd2a and full length NMNAT1 (50).

The protective mechanism of the *Wld_s* protein is not fully understood. In neurons, *Wld_s* predominantly localises to the nucleus where it activates SIRT1, leading to changes in gene expression (51). However, a small proportion of *Wld_s* is found in axons and the presence and activity

of this axonal pool affords protection against degeneration (52). Furthermore, the neuroprotective properties of a recombinant NMNAT1 fused to an axonal targeting peptide (53), along with the finding that NAD depletion triggers axonal degeneration (54), provided supporting evidence that the key to axonal protection is the NMNAT1 activity by the Wld_s protein.

Recent work has established NMNAT2 as a critical factor for maintaining axonal integrity. In neurons, NMNAT2 is subject to continuous anterograde transport necessitated by the short half-life of the enzyme. Blocking this transport triggers Wallerian degeneration (Figure 3) (55,56). Due to the protective effect of Wld_s in several disease models (57-59), the expression level of NMNAT2 has been proposed to play a role in several neurodegenerative disorders. Indeed, reduced levels of NMNAT2 have been linked to decreased cognitive function in humans and tauopathy in mice (60,61). However, the role of NMNAT2 in these conditions is not understood.

The mechanisms underlying axonal degeneration downstream of NMNAT2 are currently debated and two different models have been proposed (Figure 3). The first model suggests maintenance of axonal NAD to be essential for survival, yet both *in vitro* and *in vivo* studies performed with CD38^{-/-} and PARP^{-/-} models excluded a contribution of these NAD-consuming enzymes in axonal degeneration (54). Recently, SARM1 (sterile alpha and Toll/interleukin-1 receptor motif containing 1) was identified as a central executioner of the axon degeneration pathway which is essential for NAD depletion in injured neurons (62,63). In *Sarm1*^{-/-} mice, axonal degeneration is delayed by several weeks following injury. In addition, these mice display improved outcomes after the induction of peripheral neuropathy and traumatic brain injury (62,64,65). Mechanistically, SARM1-mediated NAD depletion was revealed by the discovery of the SARM1 TIR domain as a member of an ancient class of NAD glycohydrolases (66,67). SARM1 NAD glycohydrolase activity is driven by dimerisation of its TIR domain, and forced dimerisation induces catastrophic NAD depletion in the absence of injury (54).

The second model proposes that the loss of NMNAT2 in injured axons leads to the accumulation of neurotoxic levels of NMN. In line with this model, axon degeneration can be stalled by inhibiting NamPRT while NAD levels are maintained by NAR supplementation (68,69). A transgenic mouse model overexpressing *E. coli* NMN deamidase provided further support for this model. The degree of axonal protection in these transgenic mice was similar to those observed in Wld_s or *Sarm1*^{-/-} mice and explained by the conversion of NMN to NAMN (70). However, the concept of NMN as a neurotoxic agent does not agree with the finding that increasing NamPRT expression is neuroprotective (71).

Sustaining NAD levels by pharmacological agents

Depletion of NAD is not only associated with neurodegeneration but also other pathologies including metabolic disorders (72,73), heart diseases (74,75), muscle atrophy (76) and renal dysfunction (77). Moreover, NAD levels decline with age in multiple tissues of rodents (73,78-80) and humans (81,82). NAD depletion is governed by two principle mechanisms - decreased NAD biosynthesis and increased NAD consumption (Figure 4).

A striking example of the deleterious physiological consequences of deficient NAD biosynthesis is pellagra, a B3 hypovitaminosis resulting from insufficient supply of dietary NAD precursors (83). NAD depletion can also be caused by reduced or impaired activity of NAD biosynthetic enzymes. For instance, NamPRT levels decrease with age (73,84) and are affected in different models of heart failure (74,75). Impaired QAPRT function was shown to predispose kidneys to acute injury owing to suppression of *de novo* NAD biosynthesis and renal NAD depletion (77).

DNA repair deficiencies implicated in neurodegenerative disorders such as xeroderma pigmentosum, ataxia-telangiectasia and Cockayne syndrome are linked to chronic PARP1 activation and increased NAD consumption (85-87). PARP1-mediated NAD depletion has also been associated with ageing due to the combined effect of reduced efficiency of the DNA repair machinery and increased levels of reactive oxygen species leading to recurring DNA lesions (78,80). Furthermore, a considerable NAD decline in multiple tissues in aged mice has been linked to CD38 over-activation (88) or results from SARM1 activation in response to nerve injury, as outlined in the previous chapter (66).

Many studies using *in vivo* models that were aimed at boosting NAD levels either pharmacologically or by supplementation with NAD precursors, have established promising therapeutic concepts for age-related diseases (Figure 4). Pharmacological inhibition of PARP1 (89-91) or CD38 (92,93) was shown to elevate NAD concentrations in several tissues. However, since these enzymes control many important signalling pathways, any pharmacological manipulation of them needs to be conducted with caution. For NAD biosynthesis enzymes, a pharmacological activator has been identified only for NamPRT. The increase in cellular NAD levels in the presence of the neuroprotective aminopropyl carbazole agent P7C3 could be attributed to the stimulation of the activity of this enzyme (94).

The concept of boosting NAD levels by dietary supplementation of NAD precursors dates back to 1937, when Conrad Elvehjem demonstrated that both Nam and NA can prevent canine pellagra (83). Dietary Nam or NA also efficiently increase the NAD content in multiple tissues of rodents (95). Nam supplementation was shown to reduce oxidative stress and inflammation in mouse models (96) and to prevent hepatosteatorosis (96), acute kidney injury (77) and glaucoma (97). However, there are limitations to the use of these dietary NAD precursors. The severe flushing induced by NA results

from activation of the G-protein-coupled receptor GPR109A (98), whereas high doses of Nam can inhibit NAD-dependent enzymes (e.g. sirtuins and PARPs).

During the last few years, a number of excellent studies have demonstrated that the supplementation with alternative NAD precursors, NR and NMN, considerably increases NAD levels and restores various physiological functions that are deteriorating in pathological conditions or during ageing. For example, both NR and NMN improve glucose metabolism and protect against obesity and type 2 diabetes (72,73,99). Furthermore, these compounds induce mitochondrial biogenesis (79,100-102), improve muscle (76,79,99), neural (86,103-105) and various other functions (for an extensive review see (106)). Dietary supplementation of NR has been associated with a slight lifespan extension in mice (107) and an increase in NAD levels in blood cells in humans (108,109). Importantly and in contrast to NA, NR is well-tolerated in humans (109).

Pathological consequences of NAD depletion in tissues may be caused by a wide variety of molecular alterations ranging from failure of energy metabolism to dysfunctions of NAD-dependent signalling processes. Therefore, it is difficult to identify the exact molecular mechanisms that lead to the restoration of the physiological functions following NAD boosting therapies. Nevertheless, a growing body of evidence suggests that one of the universal mechanisms for the beneficial health effects of NAD replenishment is activation of Sirtuins (51,73,79,86,90,102,106).

Conclusions and perspectives

The importance of the regulatory roles of NAD has been firmly established in several excellent studies. While impressive progress has been made regarding the mechanisms of NAD-dependent signalling, some critical questions remain unanswered. For example, NAD demand and turnover vary greatly between tissues and cell types, and, as a consequence, biosynthesis of the dinucleotide needs to be fine-tuned. However, little is known about the regulation of NAD biosynthesis and its interplay with signalling pathways. Moreover, cellular NAD biosynthesis and consumption are compartmentalised in the cell, but how these organellar NAD pools are established and maintained is not fully understood. Mitochondria are of particular interest because they represent an important hub of NAD dependent signalling and probably contain the largest intracellular NAD pool. How this pool is sustained is debated and both autonomous biosynthesis and import of the dinucleotide have been proposed.

Impaired biosynthesis and increased NAD consumption are associated with ageing and different pathologies such as degeneration of axons in injured neurons. Understanding the exact mechanism underlying the role of NAD and NMN in axonal degeneration will lead to new therapeutic concepts to

support nerve regeneration. Increasing NAD biosynthesis by providing precursors may also be a promising therapeutic approach for other pathological states including type 2 diabetes, obesity, metabolic disorders and heart disease. Unfortunately, in the majority of the studies establishing these beneficial effects only one precursor was used, therefore it is impossible to judge the relative efficiencies of the different agents or their advantages and possible disadvantages. Moreover, the dosages used in the different studies were most often in a rather narrow range. Thereby, an important task for future research would be to establish the most efficient NAD precursor supplementation scheme and the determination of optimal dosages.

Finally, the discovery of SARM1 as a NADase and its striking role in axon degeneration has added an unexpected aspect in the understanding of NAD homeostasis. Given that another NADase, CD38, plays a critical role in age-dependent NAD decline, it appears that NADases may have a much more severe impact on the NAD metabolome as so far appreciated. Therefore, these enzymes represent attractive targets for the maintenance of physiological NAD levels.

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Figure legends

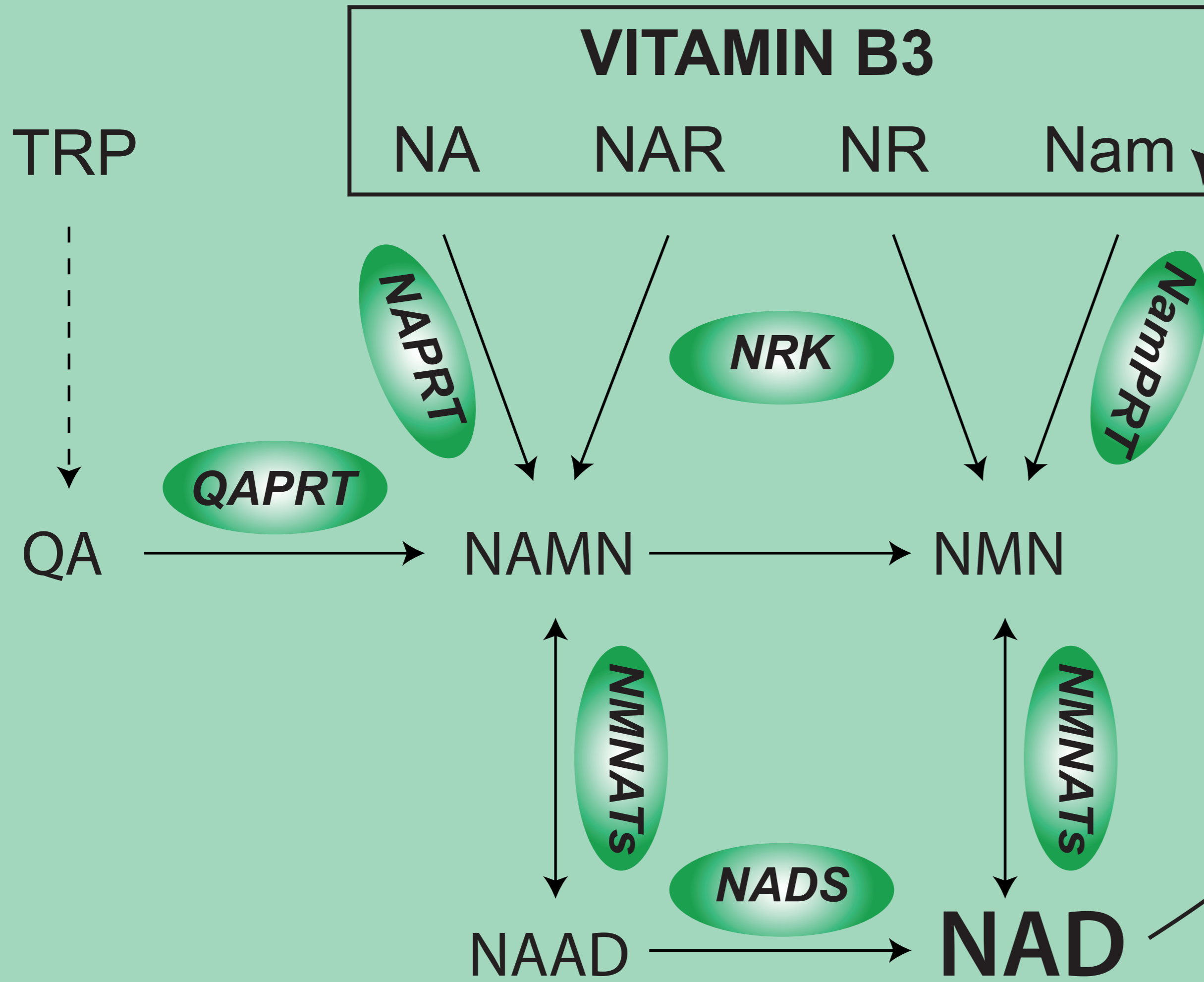
Figure 1. Metabolism of NAD in humans. NAD biosynthesis requires two major steps, the first comprises mononucleotide formation of NMN or NAMN catalysed by phosphoribosyltransferases (PRTs) specific for nicotinamide (Nam), nicotinic acid (NA) or quinolinic acid (QA) and by nicotinamide riboside kinases (NRKs) phosphorylating both nicotinamide riboside (NR) and nicotinic acid riboside (NAR). The second step is dinucleotide generation via condensation of NMN/NAMN with the AMP moiety of ATP by nicotinamide mononucleotide adenylyltransferases (NMNATs). Mononucleotide precursors are collectively referred to as vitamin B3 except for QA which is a product of tryptophan catabolism. NAD formation from NAMN requires amidation of nicotinic acid adenine dinucleotide (NAAD) by NAD synthetase (NADS). Phosphorylation of NAD by NAD kinase (NADK) leads to generation of NADP. NAD and NADP partake in redox reactions, where they are reversibly reduced to NADH and NADPH. NAD is consumed by mono- and poly-ADP-ribosylation catalysed by members of the PARP/ARTD family of proteins, by sirtuin mediated protein deacylation leading to the release of O-acylated-ADP-ribose (OAADPR) and by synthesis of the calcium releasing second messengers cyclic ADP-ribose (cADPR), ADP-ribose (ADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP). Release of nicotinamide is common to all NAD-consuming processes, and nicotinamide salvage through NamPRT therefore constitutes the most important NAD biosynthetic route.

Figure 2. Compartmentalisation of NAD metabolism and biosynthesis. All NAD biosynthetic enzymes involved in the formation of the pyridine mononucleotides NMN and NAMN display nuclear-cytosolic localisation (exemplified for NamPRT), whereas three isozymes of NMNAT localise to the nucleus (NMNAT1), the mitochondria (NMNAT3) and the Golgi apparatus facing towards the cytosol (NMNAT2). Localisation of major NAD consuming reactions carried out by, for instance, members of the Sirtuin and PARP family of proteins overlaps with those found for NMNATs. The role of NMNAT3 in mitochondrial NAD biosynthesis is elusive. For NMNAT3-dependent mitochondrial NAD biosynthesis, the apparent absence of mitochondrial NMN producing enzymes requires the presence of a hitherto unknown NMN transporter. An NMNAT3-independent route for mitochondrial NAD demands for the existence of a hitherto unidentified NAD carrier. Evidence for NAD transport across a biological membrane has so far been provided only for peroxisomes. Pyridine nucleotides are supposed to be freely exchangeable between the nucleus and the cytosol, however, substrate competition between nuclear NMNAT1 and cytosolic NMNAT2 may regulate NAD availability in these two compartments.

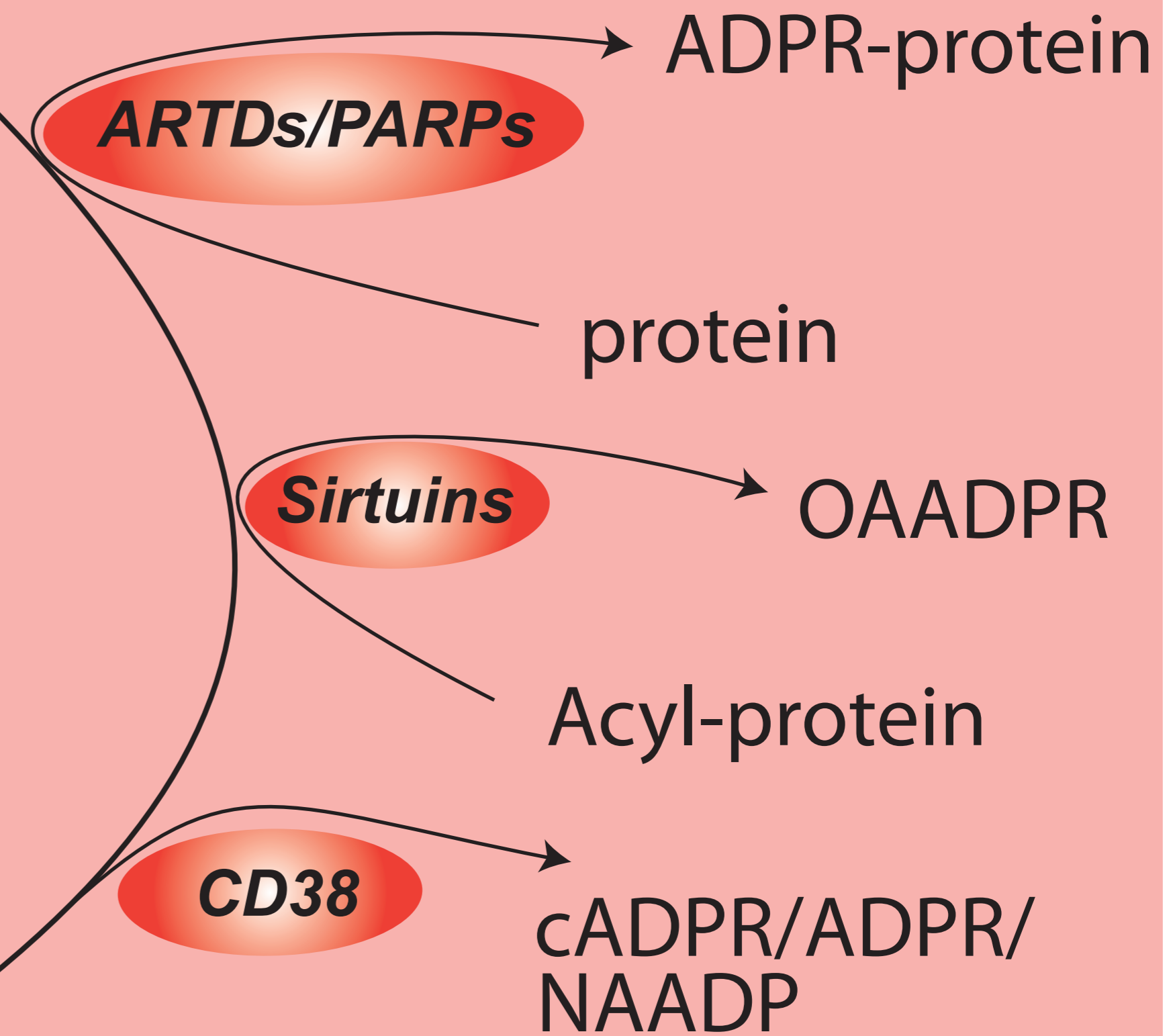
Figure 3. The role of NAD metabolism in maintaining axonal integrity. (A) In healthy neurons, NMNAT2 is transported to the distal nerve ends and maintains NAD levels. (B) Upon axotomy, NMNAT2 is rapidly degraded. The subsequent loss of axonal integrity is currently explained by two models: According to the first model, induction of SARM1 NAD glycohydrolase activity leads to catastrophic NAD depletion, whereas the second model suggests NMN accumulation to be neurotoxic. Supporting evidence for these models has been provided by the following observations: Degeneration of injured axons is delayed by (C) expression of *Wld_s*, encoding a mutant protein composed of full length NMNAT1 fused to an N-terminal portion of the ubiquitin ligase Ufd2a, (D) knockout of *Sarm1*, (E) heterologous expression of *E. coli* NMN deamidase, and (F) supplying nerve cells with a combination of the NamPRT inhibitor FK866 and NAR, an NAD precursor that is not dependent on NamPRT activity.

Figure 4. Sustaining NAD levels by pharmacological agents. Tissue NAD levels decrease with ageing and under various pathological conditions. NAD depletion can be the result of decreased NAD biosynthesis, dietary deficiency of NAD precursors, inhibition of NAD biosynthetic enzymes, and chronic activation of NAD⁺ consuming enzymes (e.g. PARP1, CD38 and SARM1). Several concepts have been developed aimed at sustaining NAD levels using pharmacological agents. These include inhibitors of NAD-consuming enzymes PARP1 and CD38, activators of NAD biosynthetic enzymes such as NamPRT, as well as supplementation with NAD precursors (e.g. NA, NAM, NR or NMN). Stimulation of sirtuin activity by NAD replenishment is one of the universal mechanisms responsible for the beneficial health effects

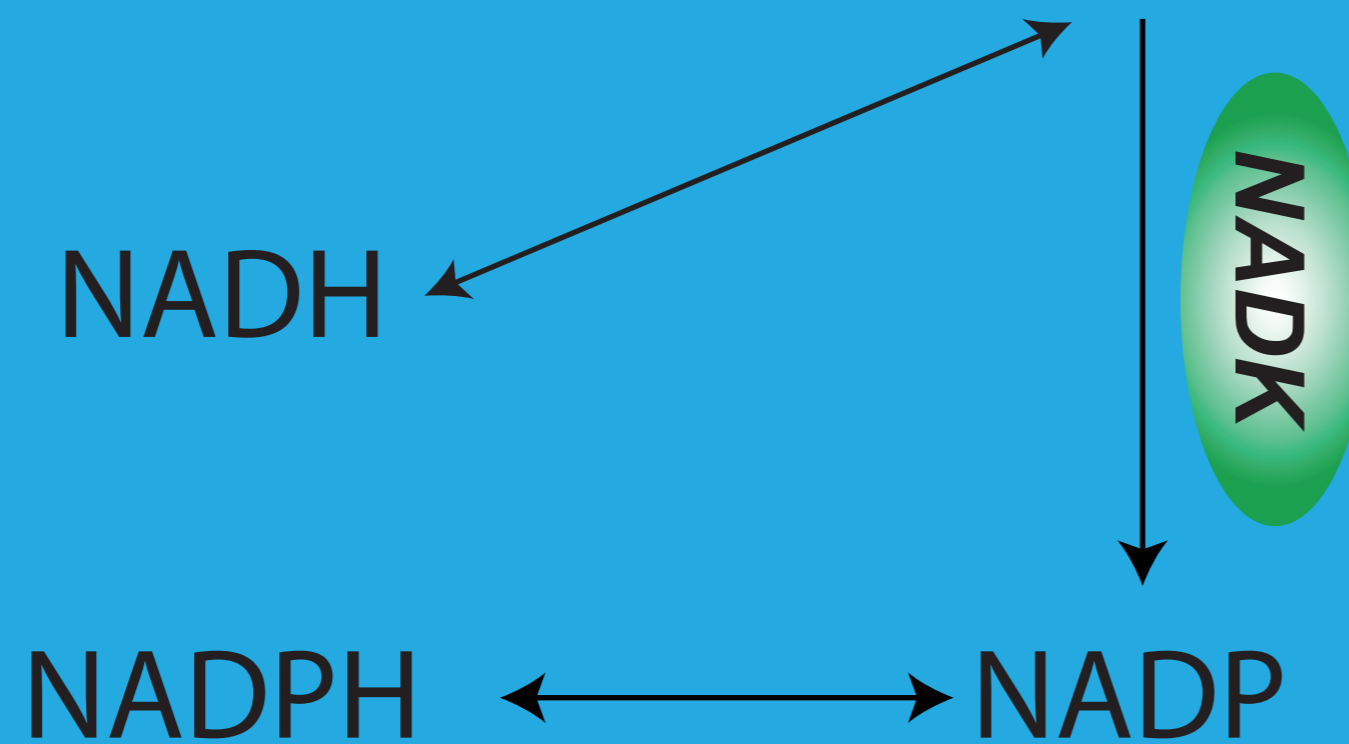
NAD biosynthesis

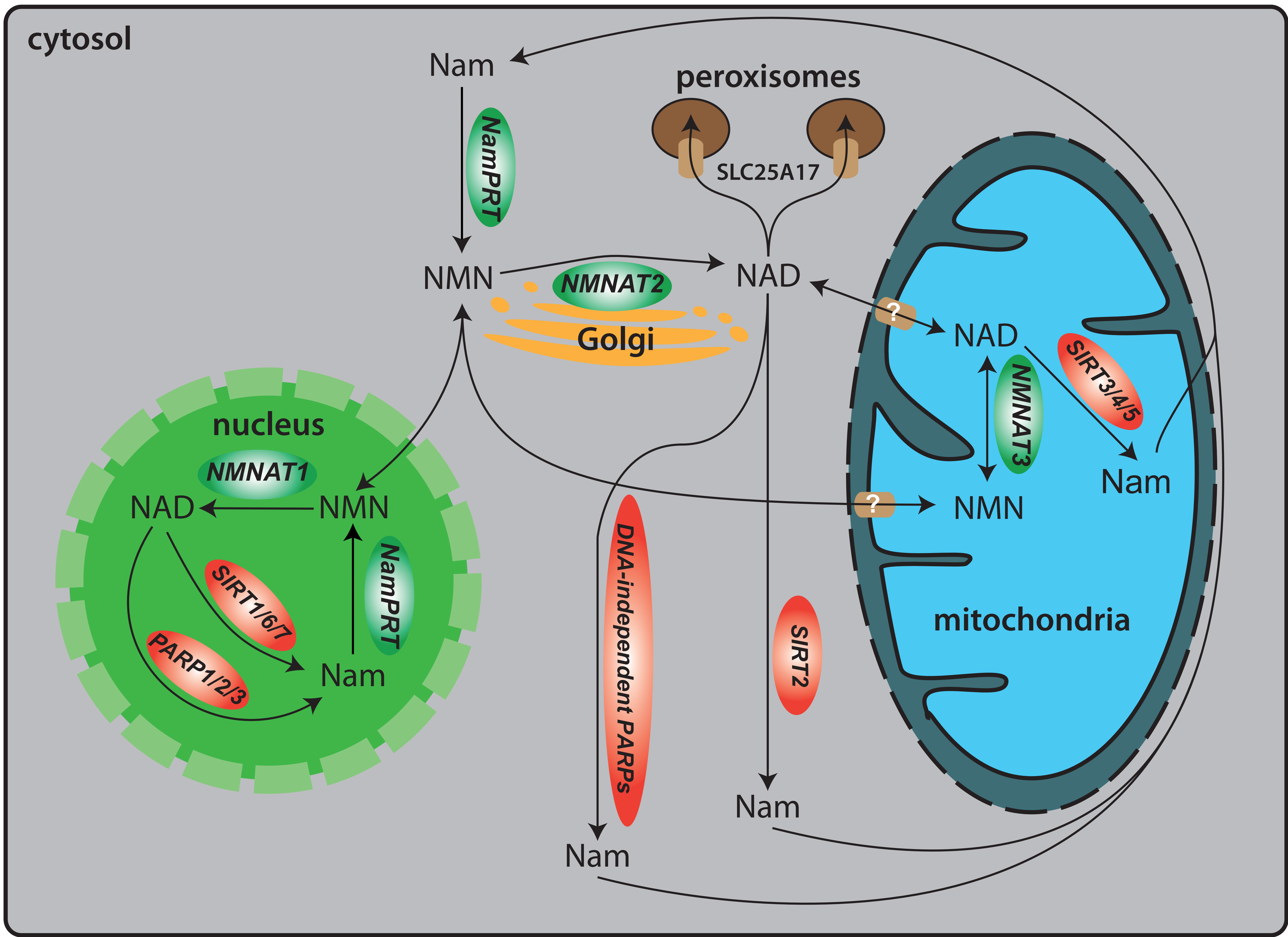


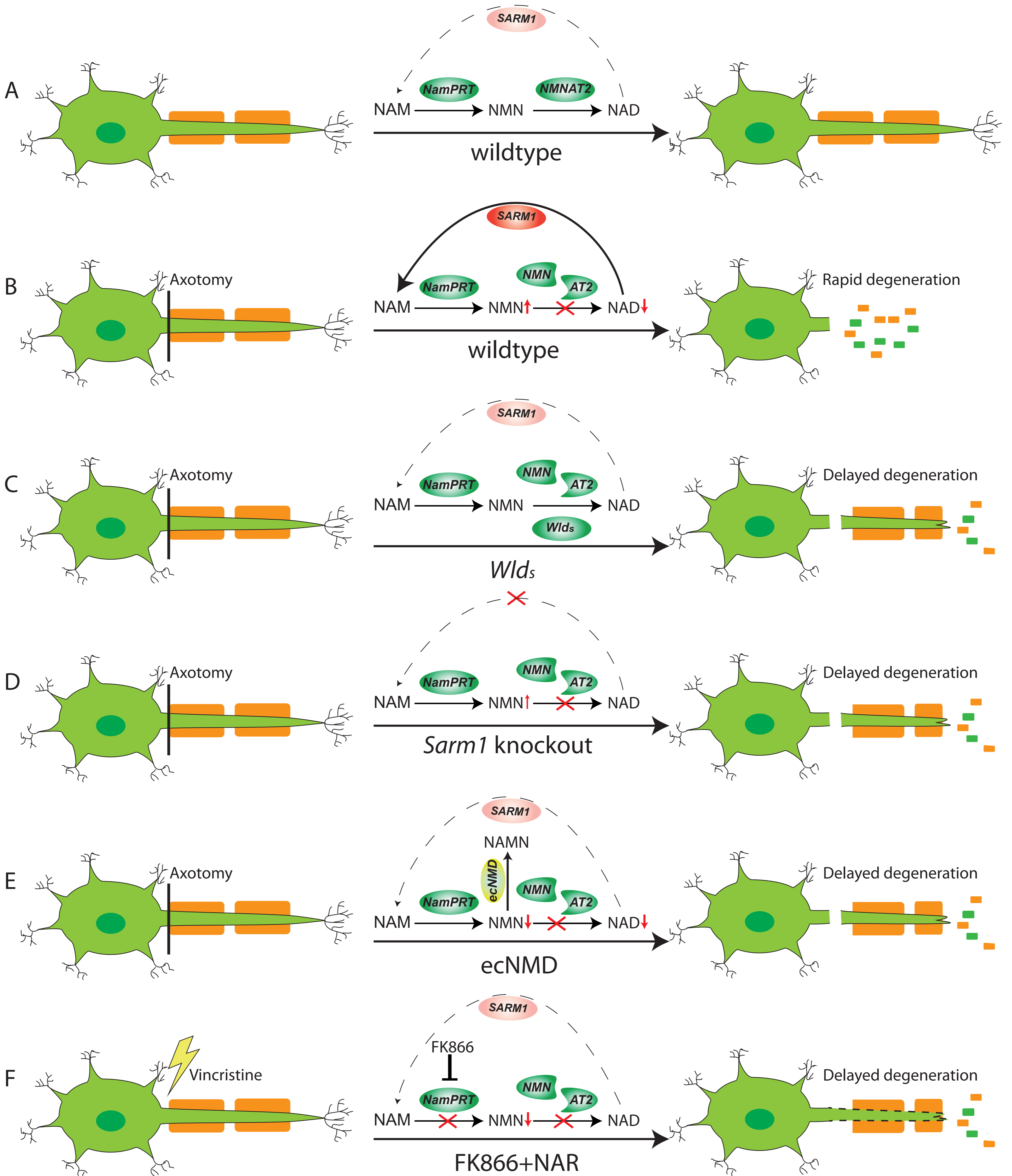
NAD-dependent signalling

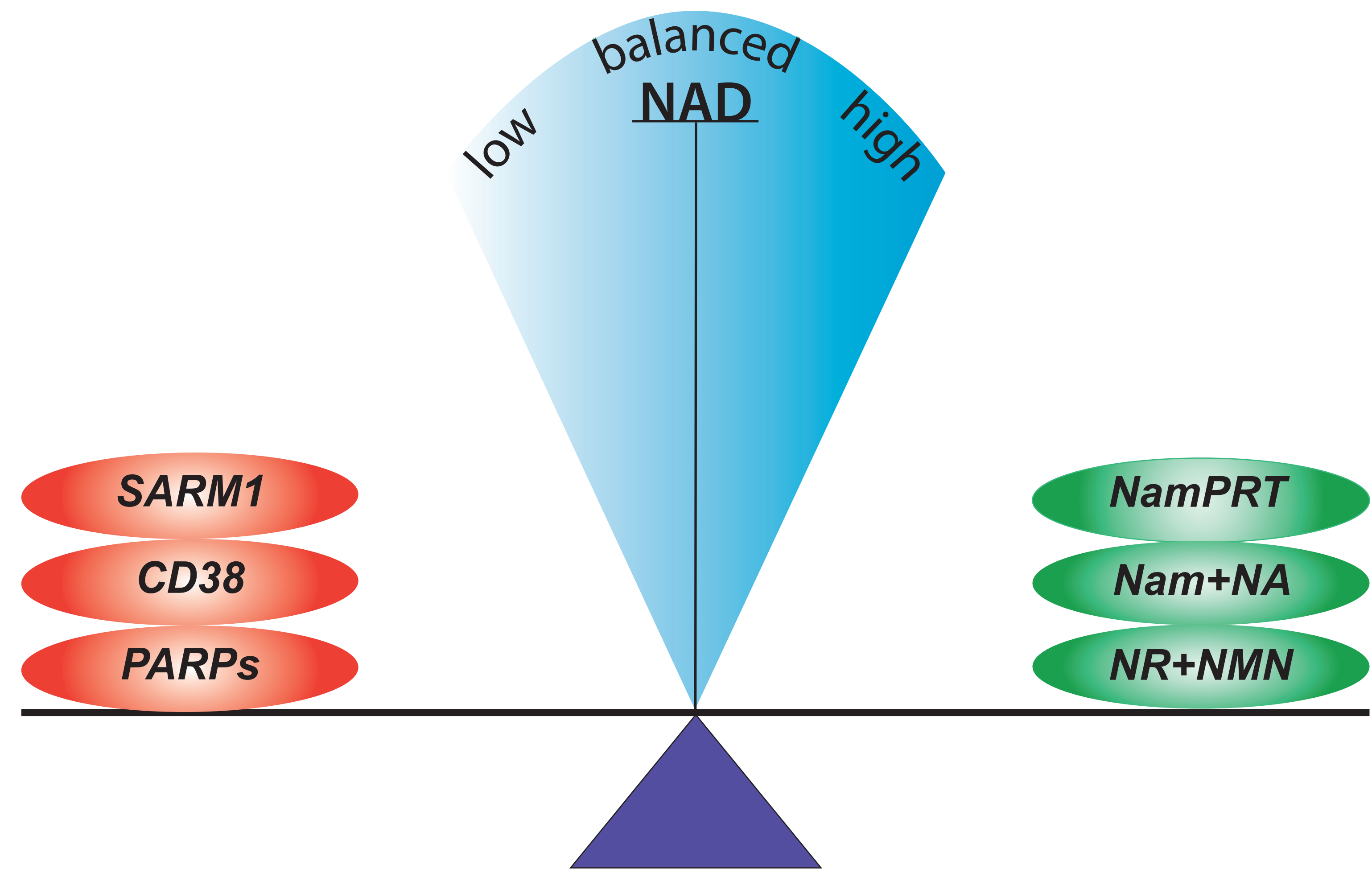


NAD dependent redox reactions









Ageing
Pathological conditions

Stimulation of NAD biosynthesis
NAD precursor supplementation

Inhibition of
NAD consuming
enzymes

