Compartmentation of NAD⁺-dependent signalling

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Abstract

NAD⁺ plays central roles in energy metabolism as redox carrier. Recent research has identified important signalling functions of NAD⁺ that involve its consumption. Although NAD⁺ is synthesized mainly in the cytosol, nucleus and mitochondria, it has been detected also in vesicular and extracellular compartments. Three protein families that consume NAD⁺ in signalling reactions have been characterized on a molecular level: ADP-ribosyltransferases (ARTs), Sirtuins (SIRTs), and NAD⁺ glycohydrolases (NADases). Members of these families serve important regulatory functions in various cellular compartments, e.g., by linking the cellular energy state to gene expression in the nucleus, by regulating nitrogen metabolism in mitochondria, and by sensing tissue damage in the extracellular compartment. Distinct NAD⁺ pools may be crucial for these processes. Here, we review the current knowledge about the compartmentation and biochemistry of NAD⁺-converting enzymes that control NAD⁺ signalling.

1. Introduction

The variety of physiological roles played by NAD⁺ has long been underestimated. Besides its vital functions as electron carrier, this pyridine nucleotide has important functions in signalling pathways [1–4]. The expression of several genes is linked to the redox state of pyridine nucleotides via NAD⁺/NADH-binding proteins that act as redox sensors [5,6]. NAD⁺ also mediates post-translational protein modifications such as ADP-ribosylation and NAD⁺-dependent protein deacetylation [7–13]. Moreover, it is a direct precursor of messenger molecules such as ADP-ribose (ADPR), cyclic ADP-ribose (cADPR) and O-acetyl-ADP-ribose (OAADPR), which are all involved in intracellular Ca²⁺ mobilization [14–16]. NAD⁺-mediated signalling has now moved into focus as it governs fundamental biological processes both on a cellular and organismal level. To fulfil such a broad range of functions a variety of mechanisms leading to signalling conversions of NAD⁺ have evolved. Several enzymes have been identified that catalyse different conversions of NAD⁺ leading to highly specific or rather general physiological consequences. This multitude of different signalling pathways requires precise spatial organization. Several excellent recent reviews have covered various aspects of NAD signalling [1–16]. This review focuses on the biochemistry and compartmentation of NAD⁺-converting enzymes and of NAD⁺ itself in mammalian cells.

2. Conversions of NAD⁺ in signalling reactions

In redox reactions, NAD⁺ is reversibly converted to NADH by addition of two electrons and a proton to the nicotinamide ring, thereby oxidizing metabolic intermediates (Fig. 1). In contrast, NAD⁺-dependent signalling reactions involve cleavage of the glycosidic bond between nicotinamide and ADP-ribose (wavy red line in Fig. 1), i.e., the consumption of NAD⁺. These reactions result in the transfer of the ADP-ribose moiety onto an acceptor, and release of the remaining nicotinamide. Therefore, these reactions are also referred to as ADP-ribosyl transfer.

2.1. NAD⁺-dependent protein modification and generation of second messengers

There are two principal types of NAD⁺-dependent signalling reactions, the modification of proteins and the generation of messenger molecules (Fig. 1). NAD⁺-dependent protein modifications include ADP-ribosylation reactions, that is, covalent attachment of ADP-ribose to specific amino acid residues, and the deacetylation of lysine residues, catalysed by ADP-ribosyltransferases (ARTs) and sirtuins (SIRTs), respectively. ADP-ribosylation and deacetylation activate or inactivate target protein functions, as in the case of other posttranslational protein modifications. ADP-ribosylation is a
NAD⁺-converting enzymes and NAD⁺-dependent signalling. In redox reactions, NAD⁺ is reversibly converted to NADH by addition of two electrons and a proton to the nicotinamide ring. NAD⁺-converting enzymes cleave the glycosidic bond in NAD⁺ linking nicotinamide to ribose (indicated by the red wave). ADP-ribosyltransferases (ART) transfer ADPR onto amino acid residues (mono-ADP-ribosylation) or onto protein-linked ADP-ribose moieties (poly ADP-ribosylation). These protein modifications are reversed by ADP-ribosylhydrolases (ARH) and poly-ADP-ribosyl-glycohydrolase (PARG), liberating ADPR. Sirtuins (SIRT) catalyse NAD⁺-dependent deacetylation of proteins carrying acetylated lysine residues, yielding O-acetyl-ADPR (OAADPR). NAD-glycohydrolases (NADase) catalyse hydrolysis of NAD⁺ to ADPR and nicotinamide and cyclisation of ADPR to cyclic-ADPR. NADases also catalyse substitution of the nicotinamide moiety in NADP⁺ by nicotinic acid, yielding NAADP. These NAD⁺-derivatives can trigger elevation of intracellular Ca²⁺.

2.2. Three NAD⁺-converting protein families

In enzymes catalysing redox reactions the adenine and pyridine moieties of NAD⁺/NADH typically each are bound by a separate classic Rossmann/Walker nucleotide-binding fold. The Cbfp family of redox-responsive transcription factors similarly binds NAD⁺/NADH with two separate Rossmann fold [17]. The enzymes catalysing NAD⁺-consumption fall into three individual protein families containing distinct NAD⁺-binding domains. SIRTs use a single copy of the Rossmann fold for coordinating the adenine moiety of NAD⁺ [18,19]. ARTs and NADases carry rather distinct NAD⁺-binding domains.

ARTs bind NAD⁺ in an extended conformation in a Pacman-like crevice formed by two abutting β-sheets [20,21]. Nicotinamide is buried in a deep pocket, exposing the glycosidic bond between nicotinamide and the adjacent ribose moiety for nucleophilic attack by a variety of substrates, including amino acids, nucleotides, ADP-ribose, phosphate, antibiotics, and water. Mammals use the ART family mainly for posttranslational ADP-ribosylation of amino acid residues in proteins and for the ADP-ribosylation of protein-linked ADP-ribose moieties, generating polymers of ADP-ribose (PARylation) [11]. One member of the ART family catalyses transfer of ADP-ribose to the terminal phosphate in t-RNA, initiating the splicing reaction and the release of ADP-ribose 1’–2’ cyclic phosphate [22,23]. The ART fold has been found in proteins from all kingdoms of life. In mammals, the ART family is the largest and, perhaps, most versatile of the three NAD⁺-converting protein families [10]. The 20–25 members of the ART family can be subdivided into two structurally distinct subclasses designated ARTC and ARTD on the basis of structural similarities to cholera and diphtheria toxins, respectively [11]. Members of the smaller ARTC subfamily are expressed as GPI-anchored or secreted ecto-enzymes. Members of the larger ARTD subfamily include nuclear poly-ADP-ribosyltransferases, the t-RNA dephosphorylating enzyme TpT, and cytosolic oligo- and mono-ADP-ribosyltransferases. SIRTs have a low intrinsic NAD⁺ binding affinity, and NAD⁺-binding to SIRTs generally requires prior binding of an acetylated peptide [18,19]. This peptide forms the central strand of a three-stranded enzyme-substrate β-sheet in a crevice between the small Zn-binding domain and the large Rossman nucleotide-binding fold. NAD⁺ binds in an extended conformation that exposes the alpha face of the nicotinamide ribose to the carbonyl oxygen of the acetyl lysine substrate promoting formation of an O-alkylamidase reaction intermediate, the deacetylation of lysine and the release of O-acetyl ADP-ribose. The SIRT fold has been found in proteins from all kingdoms of life [24,25]. The seven members of the mammalian Sirtuin family are located in the nucleus, cytosol, and mitochondria [24].

NADases bind NAD⁺ in a bent conformation [26,27]. These enzymes facilitate base-exchange of the nicotinamide moiety, intramolecular cyclisation or hydrolysis of the high energy bond. The NADase fold has been identified so far only in proteins of metazoan animals [16]. In mammals, the two members of the ADP-ribosylc-class/NADase family are expressed as membrane bound ecto-enzymes [16,28].

Some members of these protein families show overlapping enzyme activities, e.g., hydrolysis of NAD⁺ by some ARTs and the ADP-ribosylation of proteins by some SIRTs.
2.3. Physiological functions of NAD*-dependent signalling processes

Over the last years, important discoveries have been made with regard to all the signalling conversions mediated by NAD*. Nevertheless, NAD*-dependent protein deacetylation by SIRT1 has run the show lately, because its activity has been associated with a number of fundamental biological processes, most prominently regulation of the circadian clock and ageing [12,24,25]. SIRT1 deacetylates histones at specific gene loci and thereby silences expression of the corresponding genes. Accordingly, activation of the SIRT1 homologues in non-mammalian species such as yeast, Caenorhabditis elegans or flies leads to substantial extension of life span. In mammals, SIRT1 has been shown to regulate the activity of important transcription factors such as p53, PGC1α, Forkhead-box proteins and others. Thereby, its activity affects metabolic homeostasis, participates in the regulation of the biological clock, and increases stress resistance. Moreover, sirtuins regulate the activity of key metabolic enzymes. In most cases, the deacetylation leads to catalytic activation of the target proteins.

The ART family also has a broad scope of regulatory functions, ranging from DNA repair to sensing cell death in inflammation [1,10,11]. Poly-ADP-ribosylation has largely been associated with nuclear events [9]. The most prominent enzyme, referred to as poly-ADP-ribose polymerase 1 (PARP1) or ARTD1 [11], has a critical function in DNA repair, based on its high affinity to DNA lesions. Under normal physiological conditions PARP1 is rather inactive, whereas its binding to DNA strand breaks dramatically increases its catalytic activity. Thereby, the site of DNA damage is “marked” with polymers of ADP-ribose, which facilitates the recruitment of DNA repair factors. PARP1 also participates in the regulation of transcription, the regulation of chromatin structure and other nuclear processes related to DNA rearrangements [9,29]. Following the release of NAD* from cells during inflammation, ARTs tethered to the extracellular leaflet of the plasma membrane ADP-ribosylate the ectodomains of numerous cell surface proteins, and secretory ARTs ADP-ribosylate inflammatory cytokines [8]. These extracellular ADP-ribosylation reactions play important regulatory roles in cellular communication and immune cell homeostasis [30,31].

Second messenger molecules generated during NAD*-dependent protein modifications or directly from NAD* or NADP* by NADases trigger the release of calcium from intracellular stores or entry of calcium through plasma membrane channels [14,15]. Cyclic ADP-ribose activates the ryanodine receptor leading to the release of calcium from the endoplasmic reticulum, similar to inositol-1,4,5-trisphosphate (IP3), which activates the IP3 receptor. In addition, cADPR is a ligand of the TRPM2 channel (a member of the transient receptor potential cation channel family) and thereby stimulates the influx of calcium from the extracellular space. Both ADPR and OAADPR have also been reported to activate TRPM2 channels. NAAADP releases calcium from intracellular, possibly cell type-specific stores by activating lysosomal calcium channels and the ryanodine receptor [32–35]. The capacity of NAD*-derived messengers, in addition to IP3, to selectively trigger calcium release into the cytosol may provide mechanisms to confine calcium signalling in time and space and to allow calcium signalling by specific mechanisms in response to different extracellular signals.

3. Compartmentation of NAD*-dependent signalling pathways

Given the variety of NAD*-degrading processes and subsets of enzymes competing for the same substrate, NAD*-dependent regulatory pathways need to be tightly regulated, e.g., by specific upstream mechanisms that govern the activation or inhibition of individual NAD*-dependent mechanisms [3,4]. Moreover, NAD*-dependent signalling pathways are compartmentalized, providing another level of regulation and sophistication. Fig. 2 depicts the cellular distribution of known enzyme activities contributing to NAD*-dependent regulatory pathways and illustrates estimated concentrations of NAD* in different cellular compartments [4]. Interestingly, a number of NAD*-dependent regulatory reactions take place on the outer surface of the plasma membrane, despite the rather low concentration of NAD* (submicromolar) in extracellular fluids, e.g., serum [2]. Below, we provide a few examples of NAD*-mediated signalling processes that take place in different subcellular compartments and on the cell surface.

![Fig. 2. Compartmentation of NAD* and NAD*-converting enzymes. In mammals, NAD*-converting enzymes have been identified in the extracellular space, the cytosol, the nucleus and mitochondria. The concentration of NAD* is high in mitochondria (~400 μM), intermediate in the nucleus and cytosol (~100 μM) and low (<1 μM) in the extracellular space, and can vary considerably depending on the cell type, metabolic condition, stress, and redox status. Mounting evidence indicates that vesicular compartments including the endoplasmic reticulum, the Golgi apparatus, exocytotic vesicles and peroxisomes also harbour NAD*, albeit at concentrations that are extremely difficult to assess experimentally (indicated in grey).]
3.1. Extracellular NAD⁺-dependent signalling

The plasma membrane is impermeable to NAD⁺. However, NAD⁺ is released from cells by lytic and non-lytic mechanisms following disruption of membrane integrity by pore forming proteins, mechanical injury or osmotic forces [30,36]. Unless repaired, large or enduring leaks in the cell membrane cause breakdown of the electrochemical potential and cell death. The leakage of NAD⁺ from dying cells may represent an ancient danger signal and a universal “molecular odorant” of dead cells. Lysis of cells in multicellular organisms often accompanies infections by microbial pathogens, e.g., during infection of cells with a lytic virus, puncture of the cell membrane by bacterial haemolysins and other microbial or endogenous pore-forming proteins, e.g., complement factors or perforins secreted by cytotoxic T cells and natural killer cells. It is perhaps not surprising, then, that metazoan immune cells have developed sensors for NAD⁺ and its metabolites in the form of ecto-enzymes, ligand-gated ion channels, and G-protein-coupled receptors (GPCRs) [8,28,37].

Several members of the ART family are expressed on the outer leaflet of the plasma membrane as GPI-anchored ecto-enzymes or as secretory proteins. In response to NAD⁺ released from cells, these enzymes ADP-ribosylate other cell surface and secretory proteins. This provides a mechanism of translating pulses of leaked NAD⁺ into longer-lasting imprints [31,36]. The ADP-ribosylation of human neutrophil peptide 1 on Arg14 and Arg24 by ARTC1 and the ADP-ribosylation of the P2X7 ion channel on Arg125 and Arg133 by ARTC2 provide well-characterized examples of cell surface and secretory proteins regulated by ADP-ribosylation [8,38–40].

NAD⁺ hydrolysis by CD38, a member of the NADase family tethered to the cell surface, is an important mechanism to limit the duration of NAD⁺-signalling in the extracellular compartment [41]. Moreover, this enzyme generates potent calcium-mobilizing second messengers, e.g., ADP-ribose and cyclic ADP-ribose. Whether and how these messengers enter the cells to elevate the cytosolic calcium remains unknown. However, on the cell surface they are also further catabolised by extracellular pyrophosphatase/phosphodiesterases (ENPP, CD203) and 5’ nucleotidase (ENT, CD73) [42], thereby generating AMP and adenosine, which can activate members of the P2Y and P1 subfamilies of GPCRs. The metabolites adenosine, nicotinamide, and phosphate can be taken up by cells and used for the regeneration of NAD⁺ [42].

Dying cells also release cytosolic enzymes. Serum levels of creatine kinase and amino-acid transferases, for example are used as indicators of muscle and liver damage, respectively. Some cytosolic enzymes involved in NAD⁺-signalling have also been detected in serum: serum levels of adenosine deaminase (ADA), the enzyme catalysing conversion of adenosine into inosine, increase in rheumatoid arthritis and other chronic inflammatory diseases [43]. NAMPT, the enzyme catalysing the rate-limiting step in NAD⁺ synthesis, reportedly functions also as a cytokine acting on B cells and neutrophils [44,45] and as an adipokine [46]. It is conceivable, though not established, that other intracellular enzymes involved in NAD⁺-signalling might be released from cells in enzymatically active form. However, the pathophysiological interplay of bona-fide extracellular NAD⁺-metabolising enzymes such as ART2 and CD38 and released cytosolic enzymes such as NAMPT and ADA still need to be explored.

Apart from the lytic release of NAD⁺ from injured or dying cells, mounting evidence indicates that NAD⁺ can also be released from cells in a regulated fashion, e.g., by reversible opening of pores formed by members of the gap-junction protein family, e.g., Connexin 43 and Pannexin 1, or by vesicular exocytosis [47]. NAD⁺ reportedly is released from motor nerve terminals upon electrical stimulation and may act as a neurotransmitter on purinergic GPCRs expressed by smooth muscle cells in blood vessels, the kidney and the gut [48,49].

3.2. Nuclear NAD⁺-dependent signalling

Prominent NAD⁺-dependent processes in the nucleus include poly-ADP-ribosylation and protein deacetylation. Besides PARP1 (ARTD1), other ARTs generating protein-bound PAR play important roles. PARP2 (ARTD2), even though catalytically less active, appears to facilitate DNA repair, similar to, or in concert with PARP1. Poly-ADP-ribosylation is also involved in the regulation of telomerase activity. The telomeric repeat binding factor 1 (TRF1) prevents access of telomerase to telomers by binding to the telomeric DNA. When activated, the ART enzyme tankyrase 1 (ARTD5) catalyses poly-ADP-ribosylation of TRF1. Thereby, TRF1 loses its DNA binding ability and telomerase gains access to extend telomers. PARP1 can modulate transcription in a similar fashion by modifying specific transcription factors that become unable to bind to their cognate sites in the DNA. PARG activity in the nucleus rapidly degrades PAR, making poly-ADP-ribosylation a rather transient modification.

The best studied nuclear sirtuin is SIRT1. Numerous studies have documented that SIRT1 deacetylates not only histones, but also critical transcription factors. Among the consequences of SIRT1-mediated transcriptional regulation is the favourable adjustment of energy metabolism. In this regard, among the major targets of SIRT1 deacetylation are PGC1α, PPARY and FOXO transcription factors. Therapeutic concepts, based on SIRT1 activation, begin to emerge which are designed to counteract, for example, diet-induced obesity, obesity-induced diabetes and metabolic syndrome [50]. Another example as to how SIRT1 activity modulates the function of an organism as a whole was provided by its identification as an important regulator of the biological clock. It has been proposed that, at least in metabolically active tissues, SIRT1 may act as a “rheostat” within the clock system and determine the epigenetic regulation of the clock system through NAD⁺-dependent deacetylation [51].

SIRT2 has been observed to reside both in the nucleus and the cytosol. Besides histones, it targets p300, a crucial protein acetylase. Interestingly, SIRT2 and p300 interact reciprocally. That is, p300 acetylates SIRT2, while SIRT2 deacetylates p300.

3.3. Cytosolic NAD⁺-dependent signalling

ADP-ribosylation of elongation factor 2, actin, rho, and Goα, catalysed by bacterial toxins that translocate into the cytosol of mammalian cells, profoundly perturb cellular functions, causing severe diseases including diphtheria, whooping cough and cholera [52,53]. These toxins may mimic endogenous regulatory processes, and biochemical studies, indeed, have detected similar enzyme activities in various tissues [54]. However, the corresponding cytosolic proteins have so far eluded molecular characterisation. Another intriguing example of cytosolic ADP-ribosylation is the activity of vault-PARP (ARTD6): this enzyme catalyses reversible oligo-ADP-ribosylation of the major vault protein, the main structural component of a huge cytosolic ribonucleoprotein complex thought to be involved in mRNA transport [55]. Characterisation of cytosolic ADP-ribosylation reactions is hampered by the ubiquitous presence of cytosolic enzymes (ARH1 and PARG) that efficiently reverse mono- and poly-ADP-ribosylation.

SIRT2 regulates adipocyte differentiation by deacetylation of FOXO1 in the cytosol [56]. Moreover, it was identified as a tubulin deacetylase [57] and thereby contributes to the regulation of microtubule-associated functions.

An unresolved problem has been the generation of NAD⁺/NADP⁺-derived calcium messengers in the cytosol. All these mes-
sengers (ADPR, cADPR, NAADP, and OAADPR) trigger the elevation of cytosolic calcium and are known to act at the cytosolic side of calcium channels present in organelar or the plasma membranes. While ADPR and OAADPR originate from reversible ADP-ribosylation or NAD$^-$-dependent protein deacetylation, respectively, the pathway of how cADPR and NAADP arise in the cytosol has remained unclear. Both known mammalian NADases, CD38 and CD157, are ecto-enzymes and no other candidate enzyme that might catalyse the synthesis of these messengers in the cytosol has been identified. Therefore, transport mechanisms have been invoked which could mediate, for example, the entry of cADPR from the extracellular space or through the membranes of endosomes following internalization of CD38. Irrespective of these uncertainties, cytosolic calcium signalling mediated by NAD$^-$/NADP-derived messengers has been intensely studied and demonstrated in a multitude of model systems: Secretory functions of pancreatic acinar cells are coordinated by NAADP and cADPR, T cell activation relies on successive cytosolic calcium elevations triggered by different messengers, and in the brain, the regulation of TRPM2 channels by NAD$^-$-derived ligands is crucial.

3.4. Mitochondrial NAD$^-$-dependent signalling

While mitochondria had been largely regarded as purely metabolic and bioenergetic workhorses, research over the last decades identified these organelles as key players in central regulatory mechanisms. It is now clear that NAD$^-$-mediated signalling within these organelles plays a fundamental role in metabolic regulation. Key components of the respiratory chain, fatty acid degradation, and ammonia detoxification are regulated by the three mitochondrial sirtuins, SIRT3, SIRT4, and SIRT5 [13]. SIRT3 has been best studied and was shown to deacetylate a number of mitochondrial proteins including enzymes of the β-oxidation pathway, glutamate dehydrogenase, and a subunit of complex I in the respiratory chain. SIRT5 accelerates the urea cycle by deacetylating carbamoylphosphate synthase 1, the rate-limiting enzyme [58]. Besides acetylation/deacetylation, glutamate dehydrogenase also appears to be regulated by mono-ADP-ribosylation mediated by SIRT4 [59]. While the ADP-ribosylation is inhibitory, deacetylation apparently releases the inhibition caused by the acetylation of enzymes [13]. Interestingly, the synthesis of acetyl-CoA, the substrate for protein acetylation, is regulated by acetylation/deacetylation of acetyl-CoA synthase (ACS). Both the cytosolic and the mitochondrial isoforms are activated by NAD$^-$-dependent deacetylation [60].

4. NAD$^+$ pools and NAD$^-$-dependent signalling

Given the multitude of NAD$^-$-degrading activities and the dynamic interconversion of NAD$^+$ and NADH, accurate determination of NAD$^+$ concentrations in tissues or cells, let alone subcellular organelles, has remained difficult. An additional obstacle to understanding the regulation of NAD$^-$-dependent signalling pathways by substrate availability is the uncertainty regarding free vs. protein-bound NAD$. Therefore, so far the knowledge about “active” NAD$^+$ concentrations has remained rather limited and reported numbers often vary widely depending on the model system, the experimental conditions, and the method of nucleotide determination. Nevertheless, there are several interesting observations regarding the relationship of NAD$^+$ pools and NAD$^-$-consuming processes (Fig. 2). Reported NAD$^+$ concentrations in tissues are most often in the submillimolar range [4]. Generally, the mitochondrial NAD$^+$ concentration appears to be higher than that measured for whole cells or tissues. Therefore, mitochondria maintain a high NAD$^+$ concentration. Accordingly, tissues with a high content of mitochondria (e.g., heart or liver) tend to have higher NAD$^+$ contents. In mammals, the mitochondrial NAD$^+$ pool is segregated from the cytosol. NAD$^+$ exchange over the inner mitochondrial membrane has been discovered in plant and yeast mitochondria [61,62], but not in animals.

Even though conclusive experimental evidence is lacking (and very hard to obtain), the cytosolic and nuclear NAD$^+$ pools are most often considered to be readily exchangeable. Therefore, it is generally assumed that the nuclear and cytosolic NAD$^+$ concentrations are similar. Whether these are valid suppositions remains to be seen. Interestingly, the known subcellular distribution of NAD$^-$-dependent signalling mechanisms coincides with the established presence of NAD$^+$ in these compartments. The biochemical functions of peroxisomes (e.g., fatty acid oxidation) also imply the presence of NAD$^+$ in these organelles. Indeed, in a recent report the presence of NAD$^+$ in peroxisomes was visualized [63]. Surprisingly, this study also established that the endoplasmic reticulum and the Golgi complex constitute NAD$^+$ pools that are likely independent of the cytosolic one. However, so far there is no information regarding the NAD$^+$ concentration in these compartments (as indicated by their grey shading in Fig. 2), nor whether their NAD$^+$ pools also participate in regulatory processes.

The versatility of NAD$^+$ conversions in the extracellular space is in apparent contradiction to the rather low NAD$^+$ concentration measured in extracellular fluids (e.g., submicromolar in plasma, see Fig. 2). It is conceivable that the overall concentration may reflect an average of high local concentrations and an otherwise nearly complete absence of NAD$^+$ in extracellular fluids. That is, spatially confined release of NAD$^+$ may have paracrine function or signal cell damage toalert repair systems.

5. Perspectives

NAD$^+$ has emerged as an important mediator of signalling both between and within cells. This nucleotide undergoes a variety of conversions leading to protein modifications or activation of ion channels with pronounced physiological effects both on the cellular and organismal level. The consumption of NAD$^+$ by different enzymes necessitates continuous resynthesis of the molecule. How NAD synthesis and consumption are affected by the subcellular localization of the enzymes participating in NAD$^+$-synthesis and NAD$^+$-conversion, and how the supply of NAD$^+$ to different cellular compartments is organized remain challenging questions. New research tools including specific antibodies, agonists, and antagonists directed against the key enzymes of NAD$^+$ synthesis and consumption will help address these questions. Moreover, these reagents may pave the way to a new generation of therapeutics for treating metabolic and inflammatory diseases in which NAD$^+$ signalling is impaired.

References


