Published in final edited form as:
Cold Spring Harb Symp Quant Biol. 2011 ; 76: 291–298. doi:10.1101/sqb.2012.76.010439.

NAD^+ as a signaling molecule modulating metabolism

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Abstract

The ability of NAD^+ to act as a metabolic cofactor and as a rate-limiting cosubstrate for many enzymes, particularly the sirtuins, has led to the identification of a pivotal role of NAD^+ levels in the control of whole body metabolic homeostasis. Bioavailability and compartmentalization of NAD^+ have become highly relevant issues that we need to understand in order to elucidate how NAD^+ acts both as a readout of the metabolic milieu and as an effector triggering appropriate cellular adaptations.

Early in the last century, four Nobel laureates, Sir Arthur Harden, Hans von Euler-Chelpin, Otto Warburg and Arthur Komberg, contributed to the discovery of nicotinamide adenine dinucleotide (NAD^+), the solution of its structure and the definition of its key metabolic functions (Berger et al., 2004). In several cellular compartments, either oxidized or reduced NAD serves in transhydrogenase reactions catalyzed by various oxidoreductase enzymes (Houtkooper et al., 2010). It was only in the last decade, however, that the full extent of NAD^+ function began to emerge with the identification of NAD^+ consuming proteins, such as the sirtuin family of deacetylases, that in turn function as metabolic regulators (Houtkooper et al., 2010). While not the only NAD^+ consumers, sirtuins share the particularity of having a rather high K_m for NAD^+ (Houtkooper et al., 2010; Imai et al., 2000a; Smith et al., 2009). Some sirtuins, such as the most widely studied SIRT1, have a K_m for NAD^+ that lies within the range of physiological changes in intracellular NAD^+ content. This suggested that, in some circumstances NAD^+ might be rate-limiting for the reaction catalyzed by the sirtuins and that, therefore, sirtuin activity might be modulated by NAD^+ availability (Imai et al., 2000b). This hypothesis was rapidly embraced by the scientific community and led to the concept of sirtuins being “metabolic sensors” whose activity was coupled to metabolic shifts manifested as changes in the NAD^+/NADH redox state. While there are still caveats in this hypothesis, it highlighted the possible nature of NAD^+, as not just a coenzyme, but also, as a messenger that can modulate cellular transcriptional responses and metabolic adaptations.

The coenzymatic activity of NAD^+, together with the tight regulation of its biosynthesis and bioavailability, positions it well to act as a metabolic monitoring system. To be true metabolic sensors, however, NAD^+ consuming enzymes must respond to physiological changes in NAD^+ levels. In this review we will try to assemble the evidence indicating that modulating NAD^+ levels might be a valuable strategy to drive metabolic adaptations and influence sirtuin activity.

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The authors declare no conflict of interests.

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Sirtuins as NAD+ sensors

The sirtuin family of deacetylases

Sirtuins are a family of NAD^+-dependent protein deacetylases that are similar to the yeast silent information regulator 2 (Sir2). Most sirtuins remove acetyl modifications of lysine residues on histones and other proteins in a reaction that, unlike the reaction catalyzed by type I and type II histone deacetylases (HDACs), consumes NAD^+, releasing NAM, O-acetyl ADP ribose, and the deacetylated substrate (Houtkooper et al., 2010).

There are 7 members in the mammalian family of sirtuin enzymes, named SIRT1-7 (Dali-Youcef et al., 2007; Michan and Sinclair, 2007). They are ubiquitously expressed and share a conserved catalytic core of 275 aminoacids. The diversity of sirtuin genes in mammals is associated with a specialization of their function and cellular localization. In line with the role of yeast Sir2 in the regulation of chromatin structure and gene expression, SIRT1, SIRT6 and SIRT7 are localized in the nuclei of cells (Michishita et al., 2005). SIRT2 is mainly localized in the cytoplasm (Michishita et al., 2005), even though it can also regulate gene expression by deacetylating transcriptional regulators that display nucleo-cytoplasmic shuttling (Jing et al., 2007) and influences chromatin compaction upon disassembly of the cell nucleus during mitosis (Vaquero et al., 2006). The remaining sirtuin members – SIRT3, SIRT4 and SIRT5 – are mitochondrial proteins (Michishita et al., 2005; Onyango et al., 2002; Schwer et al., 2002). Additional complexity is added by the fact that some sirtuins, like SIRT1, can shuttle between compartments in response to diverse stimuli (Tanno et al., 2007). The existence of different sirtuins in a similar compartment might also be explained because not all members show similar enzymatic activity. This way, SIRT1 and SIRT5 act exclusively as deacetylases (North et al., 2003; Vaziri et al., 2001), whereas SIRT2, SIRT3, SIRT4 and SIRT6 may also act as a mono-ADP-ribosyl transferases (Haigis et al., 2006; Liszt et al., 2005; North et al., 2003; Shi et al., 2005). In the case of SIRT7 no clear enzymatic activity has been reported to date, although it has been proposed to act as a deacetylase enzyme (Vakhrusheva et al., 2008).

In the last decade sirtuins have come into the spotlight due to their ability to modulate metabolic function and lifespan in lower organisms. In general, sirtuins become active in situations of nutritional / energetic deficit and prompt whole body metabolic adaptations aimed to improve metabolic efficiency and enhance the use of non-carbohydrate energy sources. For example, SIRT1 activation enhances lipid oxidation and mitochondrial biogenesis gene expression, whereas SIRT3 is a key regulator of acetate, ketone body and fatty acid metabolism by deacetylating mitochondrial proteins. Another sirtuin, SIRT6 negatively regulates glycolysis. For an extended view on the function of the different sirtuins, we refer the reader to some of the reviews that cover that topic (Houtkooper et al., 2010; Verdin et al., 2010; Yamamoto et al., 2007). Diverse genetic and pharmacological approaches suggest that sirtuin activation might be useful to manage metabolic and age-related diseases, generally characterized by decreased mitochondrial function (Baur et al., 2006; Herranz and Serrano, 2010; Lagouge et al., 2006). The identification sirtuin-activating compounds has, however, led to controversial results, as several of them might act through indirect means (Pacholec et al., 2010). The particularity that sirtuins require NAD^+ for their enzymatic reaction raised the interesting possibility to modulate sirtuin activity by altering NAD^+ metabolism.

Do sirtuins act as NAD+ sensors?

The concept that sirtuins could act as NAD^+ sensors has its roots in two observations in yeast. First, that the activity of Sir2 is NAD^+-dependent (Imai et al., 2000a). Second, that Sir2 could play a key role in mediating the effects of glucose restriction on yeast replicative

lifespan (Kaeberlein et al., 1999). Connecting the dots, the Guarente lab proposed that the metabolic changes induced by calorie or glucose restriction, mainly manifested as a proportionally higher prominence of mitochondrial respiration, may lead to higher NAD^+ levels, prompting Sir2 activation (Lin et al., 2002). The activation of Sir2 would then allow the cell to properly adapt to nutrient scarcity and promote enhanced lifespan.

A few aspects of the above hypothesis are still controversial (see (Canto and Auwerx, 2009) for review) and merit some scrutiny. Amongst them, whether sirtuin activity can really respond to changes in intracellular NAD^+ levels. One premise, at least, must be met in that context, i.e. that the K_m of the sirtuins for NAD^+ falls into the physiological range of NAD^+ bioavailability. Direct experimental evidence supporting this point is, in some cases, however, preliminary or absent.

An initial difficulty to establish whether the K_m of the sirtuins is within the physiological range of NAD^+ bioavailability resides in the difficulty to properly quantify NAD^+ content itself. Through different methodologies, the intracellular NAD^+ concentrations have been estimated between 0.2-0.5 mM (for review see (Houtkooper et al., 2010; Sauve et al., 2006)). These levels, however, do not discriminate free and protein-bound NAD^+. Similarly, this approximation does not take into account the existence of cellular compartmentalization of NAD^+. For example, it has been postulated that up to 75% of total intracellular NAD^+ in heart and myocytes would be in the mitochondrial compartment (Di Lisa and Ziegler, 2001). This led to the hypothesis that mitochondrial NAD^+ levels are much higher than in other compartments. However, the evidence supporting such statement is weak at best. For example, most NAD^+ is concentrated in the cytosol in other cell types, such as hepatocytes (Tischler et al., 1977). In human erythrocytes, which have no mitochondria, NAD^+ contents averages 350 μM (Yamada et al., 2006), which is similar to the NAD^+ content measured in mitochondria-containing cell types. Mitochondrial NAD^+ concentrations have rarely been accurately determined until recently, when they were established at ~250 μM in cultured HEK293 cells (Yang et al., 2007a). In the same study, total NAD^+ concentrations in HEK293 cells were estimated at ~365 μM, (Yang et al., 2007a), suggesting that NAD^+ levels are more evenly distributed in cells than originally expected. Furthermore, accurate measurements of NAD^+ in other specific cellular compartments are missing, although preliminary data indicate that free unbound NAD^+ concentration in the nucleus might be, at least, around 70 μM (Fjeld et al., 2003). Despite the fact that current data suggest that NAD^+ levels might be evenly distributed in the cell, it is important to underscore that we still have fragmentary knowledge on the real bioavailability of NAD^+ for sirtuins.

A second major point is that the K_m for NAD^+ of different sirtuins is still far from established. In the case of SIRT1, the K_m is generally accepted to be in the 100-200 μM range, even though values between 50 and 500 μM have been reported (Houtkooper et al., 2010; Pacholec et al., 2010; Sauve et al., 2006; Smith et al., 2009; Wood et al., 2004). For SIRT2, the K_m for NAD^+ has also been set around 100 μM (Smith et al., 2009). For the mitochondrial sirtuins, the K_m of SIRT3 for NAD^+, around 300 μM (Jin et al., 2009), seems to be higher than that of SIRT5 (100 μM) in vitro (Nakagawa et al., 2009). For the other sirtuins, the values are yet to be examined. In general, the K_m of sirtuins for NAD^+ seems to fall into the physiological range of NAD^+ levels, suggesting that NAD^+ availability might in fact be rate-limiting for the sirtuin reaction. Importantly, the physiological concentrations of intracellular NAD^+ rarely fluctuate more than 2-fold (Canto et al., 2009; Chen et al., 2008; Fulco et al., 2008; Rodgers et al., 2005), which is a likely range to impact on sirtuin activity. Furthermore, the complete understanding of NAD^+ partitioning and availability will be crucial to fully determine which range of NAD^+ fluctuations are required to influence the activity of different sirtuins.

Strategies aimed to modulate sirtuin activity through NAD^+

(I): NAD^+ salvage and biosynthesis

One strategy to test how enhancing NAD^+ levels regulates sirtuin activity consists in boosting NAD^+ synthesis by supplementation with NAD^+ precursors. Different precursors can induce NAD^+ synthesis. The primary *de novo* synthesis of NAD^+ generally initiates from tryptophan (Rongvaux et al., 2003). Nicotinic acid (NA) is another NAD^+ precursor and it is transformed into NAD^+ through the Preiss-Handler pathway, therefore converging with the synthesis of NAD^+ from tryptophan. However, it is assumed that perhaps the principle source of NAD^+ comes from salvage pathways from other adenine nucleotide metabolites (Rongvaux et al., 2003). The main NAD^+ precursors that funnel through the salvage pathways are nicotinamide (NAM) and, the more recently described, nicotinamide riboside (NR). NAM generates NAD^+ through a completely independent pathway, in which the rate-limiting enzyme nicotinamide phosphorybosyltransferase (Nampt), transforms NAM into nicotinamide mononucleotide (NMN), which on its turn is converted into NAD^+ by the NMN adenylyltransferase (NMNAT) (Revollo et al., 2004). Finally, NR is phosphorylated upon its entry in the cell by the NR kinases (NRKs), generating NMN, which is then converted to NAD^+ by NMNAT (Bieganowski and Brenner, 2004).

Few studies to date have described how these precursors impact on NAD^+ levels and in particular affect sirtuin activity. Both NA and NAM can lead to higher NAD^+ levels, even though the effects might be tissue-specific. For example, NAM seems to be a more stable NAD^+ precursor in the liver upon intraperitoneal injection (Collins and Chaykin, 1972), but NA seems to be a more efficient in the kidney (Hara et al., 2007). Cell-based experiments also support the existence of cell-specific differences. For example, in NIH3T3 cells concentrations of up to 5 mM NAM were unable to increase intracellular NAD^+ levels (Revollo et al., 2004). NAM also proved to be a relatively unefficient NAD^+ precursor in HEK293 cells, but 5 mM concentrations managed to double intracellular NAD^+ (Hara et al., 2007). In the latter study, it was also proven that in human cells NA seems to be a more efficient NAD^+ precursor than NAM, as concentrations as low as 20 μM were enough to double NAD^+ content. The cell/tissue specific efficiencies of distinct NAD^+ precursors might be consequent to the differential expression of the rate-limiting enzymes in their respective metabolic pathways. This way, the metabolism of NA into NAD^+ is rate-limited by the NA phosphoribosyltransferase (NAPT), which is highly expressed in some tissues, like liver or kidney (Hara et al., 2007). A reduction in NAPT activity in HEK293 cells does not affect basal NAD^+ levels, but impairs NA-induced NAD^+ accumulation (Hara et al., 2007). To date, however, no clear link between NA supplementation and sirtuin activity has been made.

In the case of NAM, it is well-known that, in mammals, Nampt rate-limits the salvage of NAD^+ from NAM (Revollo et al., 2004). Nampt is unevenly distributed among tissues. For example, its expression is high in brown adipose tissue, liver and kidney, while its levels are low in white adipose tissue or skeletal muscle and apparently absent in brain and pancreas (Revollo et al., 2007). These findings suggest that, as happened with NA, the activity of Nampt-mediated NAD^+ biosynthesis might vary widely among different tissues The modulation of Nampt activity has a clear impact on NAD^+ levels in virtually any mammalian. cell tested to date (for examples, see (Fulco et al., 2008; Pittelli et al., 2010; Rongvaux et al., 2008; van der Veer et al., 2005)). In most of these cases, the alterations in NAD^+ levels promoted by differential Nampt activity were associated changes in SIRT1 activity (Fulco et al., 2008; Revollo et al., 2007; van der Veer et al., 2007; van der Veer et al., 2005). A complication from these experiments is that the influence that Nampt exerts on SIRT1 activity may not derive only from altering NAD^+ availability, but also from NAM clearance. The reaction catalyzed by SIRT1, and, in general, all sirtuins, is efficiently

inhibited in a non-competitive fashion by NAM (Bitterman et al., 2002). This way, the possible accumulation of NAM upon a reduction in Nampt activity might by itself be enough to reduce sirtuin activity. As in the case of NAD^+, the current methodologies to measure NAM are rather limited and most studies to date do not report NAM levels. Different to NAD^+, NAM can freely diffuse through membranes (van Roermund et al., 1995), making it even more challenging to establish its bioavailability.

The effects of NR, the more recently discovered precursor, remain also still largely unknown. While there is already evidence that NR can increase intracellular NAD^+ in mammalian cells (Yang et al., 2007b), whether this can influence sirtuin activity is still unknown. However, several lines of evidence established in yeast suggest that this might in fact be the case. Exogenous supplementation of NR to yeast enhanced Sir2-dependent repression of recombination, improved gene silencing and extended replicative lifespan (Belenky et al., 2007). All these actions were completely dependent of NAD^+ synthesis (Belenky et al., 2007). While genetic manipulations aimed to alter the enzymatic capacity of the NAD^+ salvage metabolism are known since some time to impact on lifespan (Anderson et al., 2002; Anderson et al., 2003), the experiments using NR constitute the first solid evidence that enhancing NAD^+ bioavailability via NAD^+ precursor supplementation can also enhance sirtuin activity and prompt beneficial metabolic and longevity actions. Furthermore, the discovery that NR is present in milk poses an interesting opportunity for food-based preventive or therapeutic interventions in NAD^+-dependent metabolism.

In contrast to our poor knowledge of how NR supplementation acts in mammals, both NA and NAM have been used since a long time in a clinical setting. Niacin, basically composed of NA and NAM, has been widely used as an efficient way to overcome situations of dietary tryptophan deficits, which, when untreated lead to NAD^+ deficiency and pellagra (Sauve, 2008). Furthermore, niacin is used to treat hypercholesterolemia, as it efficiently decreases VLDL synthesis, lowers LDL cholesterol levels, while concurrently increasing HDL cholesterol (Altschul et al., 1955; Karpe and Frayn, 2004). The use of niacin as a lipid lowering agent might also result in beneficial effects on coronary disease and type 2 diabetes mellitus. Many of these beneficial actions of niacin in mice and humans - as well as undesired effects, such as spontaneous flushing - have been attributed to the activation of a putative NA-activated G-coupled receptor, GPR109A (Benyo et al., 2005; Tunaru et al., 2003). Whereas the role of GPR109A in mediating niacin-induced flushing is rather well-established (Benyo et al., 2005), the hypothesis that the therapeutic efficacy of niacin is mediated by GPR109A activation needs to be revisited. First, even if GPR109A has a relatively high affinity for NA (EC50 ~100 nM), such levels of NA are rarely found in plasma unless pharmacology primed (Kirkland, 2009), indicating that the activation of GRP109A by NA is probably fortuitous, but not biologically relevant in the basal state. Second, many of the beneficial effects of niacin, such as the lipid lowering effects (Kamanna and Kashyap, 2008), take place at higher concentrations than those required for GPR109A activation, but which lead to intracellular NAD^+ accumulation (Jackson et al., 1995). A third issue is that the specific tissue distribution of GRP109A receptors makes it very difficult to explain how niacin can perform some of its actions in particular tissues, especially in liver cholesterol homeostasis (Li et al., 2010). It is therefore tempting to speculate that some of these effects might be achieved through an NAD^+-induced activation of sirtuins and the consequent deacetylation of, for example, SIRT1 targets, such as the peroxisome-proliferator activated receptor coactivator 1 family of transcriptional coactivators (Kelly et al., 2009; Rodgers et al., 2005) and members from the sterol regulatory element-binding protein (SREBP) transcription factor family, which act as critical regulators of fatty acid, lipid and sterol homeostasis in eukaryotes (Walker et al., 2010). An additional appealing possibility to explain the effects of niacin involves the fact that it leads to adiponectin release from the white adipose tissue (Vaccari et al., 2007; Westphal et al.,

2007). Adiponectin then activates AMP-activated protein kinase (AMPK) in muscle and liver tissues and can in this way enhance NAD^+ content and SIRT1 activity (Canto et al., 2009; Iwabu et al., 2010).

(II): NAD^+ consumption

Another attractive strategy to modulate NAD^+ levels and favour sirtuin activity resides in the modulation of the activity of non-sirtuin NAD^+-consuming enzymes. There are two major families of alternative NAD^+ consumers: the poly(ADP-ribosyl) polymerases (PARPs) and the cADP-ribose synthases (CD38 and CD157) (Houtkooper et al., 2010).

PARPs use NAD^+ as a substrate for a cellular process in which the ADP-ribose moiety is not transferred to an acetyl group, as happens with sirtuins, but to build ADP-ribosyl polymers onto acceptor proteins (Chambon et al., 1963; Krishnakumar and Kraus, 2010). PARP activity is dramatically enhanced upon DNA damage and oxidative stress. Most PARP activity upon oxidative damage is driven by PARP-1, except for a residual 5-10%, which is likely to be accounted by PARP-2 (Ame et al., 1999; Shieh et al., 1998). Overactivation of PARP-1 upon oxidative damage rapidly depletes intracellular NAD^+ levels (Goodwin et al., 1978; Skidmore et al., 1979). In line with the hypothesis that NAD^+ might be rate-limiting for SIRT1 action, SIRT1 activity is downregulated in situations of PARP-1 activation (Bai et al., 2011b; Pillai et al., 2005). Interestingly, SIRT1 does not seem to be directly regulated by PARP activity (Bai et al., 2011a; Bai et al., 2011b). These observations indicate that PARP-1 and SIRT1 activity are connected through competition for a limited NAD^+ pool. Importantly, PARP-1 has a very low K_m (~20 μM) and a relatively high V for NAD^+ max (Mendoza-Alvarez and Alvarez-Gonzalez, 1993), indicating that PARP-1 activity might limit SIRT1 action, but not the other way round.

In a recent report our lab demonstrated how a downregulation of PARP-1 activity favours SIRT1 activation (Bai et al., 2011b). Genetic and pharmacological approaches aimed to abrogate PARP-1 activity lead to higher NAD^+ levels. While PARP activity is generally accepted to be rather low in the basal state, recent evidence indicates that it naturally fluctuates in a circadian fashion (Asher et al., 2010). In line with the existence of basal PARP activity, inhibition of its activity gradually leads to a build up of NAD^+ levels in cultured cells (Bai et al., 2011b). Similarly, tissues from PARP-1 knock-out mice display higher NAD^+ levels (Bai et al., 2011b), roughly 2-fold higher than wild type littermates. The increase in NAD^+ levels promoted by PARP inhibition leads, both *in vivo* and *in vitro*, to higher SIRT1 activity (Bai et al., 2011b). This way, PARP inhibition associates with the induction of the expression of genes involved in mitochondrial and lipid oxidation in a SIRT1-dependent manner. From a physiological perspective the better metabolic fitness derived from SIRT1 activation in situations of low PARP-1 activity offers the mice protection against the onset of metabolic disease in the context of diet-induced obesity (Bai et al., 2011b). Furthermore, this opens up the perspective on a possible role of PARP in ageing, as SIRT1 activity has been positively associated with the beneficial effects of calorie restriction on health- and life-span (Canto and Auwerx, 2011). Therefore, PARP inhibition could constitute a useful tool to activate SIRT1 and mimic the calorie-restricted state. In line with this hypothesis, it was recently reported that PARP activity is higher in aged tissues, leading to decreased SIRT1 activity (Braidy et al., 2011) (L. Mouchiroud, C. Canto and J. Auwerx, unpublished observations). Further confirming the hypothesis that higher PARP-1 activity might be detrimental for SIRT1 activity and global metabolism was the fact that mice expressing and additional copy of the human PARP-1, have a reduced median lifespan, impaired glucose metabolism and higher susceptibility to age-related diseases (Mangerich et al., 2010).

Interestingly, the NAD^+ boosting effects of PARP inhibition enhance the activity of SIRT1 (nuclear), but not that of SIRT2 (cytoplasmic) or SIRT3 (mitochondrial) (Bai et al., 2011b). The major difference between these three sirtuins is their subcellular localization, suggesting the existence of compartment specific NAD^+ pools in the cell. This was nicely illustrated by the Sinclair lab, showing the existence of independently regulated NAD^+ pools and that depletion of the cytosolic/nuclear pools can happen while mitochondrial pools remain intact (Yang et al., 2007a).

Another small family of NAD^+ consumers are the cADP-Ribose Synthases, CD38 and the less characterized CD157. CD38 and CD157 are multifunctional enzymes that use NAD^+ as substrate to generate second messengers, such as cADP-ribose, which regulates calcium mobilization (Lee, 2006). Most studies on how cADP-ribose synthases impact on NAD^+ levels and SIRT1 activity have been done with CD38. As happened with PARP-1, CD38 displays a very low K_m for NAD^+ (15-25 μM) (Cakir-Kiefer et al., 2001). Importantly, the stoichiometry of the reaction catalyzed by CD38 involves a massive amounts of NAD^+, around 100 molecules, to yield a single cADP-ribose (Dousa et al., 1996). Therefore, even little CD38 activity might have a dramatic influence on intracellular NAD^+ metabolism. Strikingly the enzymatic activity of CD38 is located outside the plasma membrane (De Flora et al., 1997), which makes it challenging to determine which is the accessibility of NAD^+ for this enzyme, as NAD^+ is only present in minute quantities outside the cell (O'Reilly and Niven, 2003). It was recently supported that CD38 might also be present in the nuclear compartment, which would then open a role for CD38 as a main intracellular NAD^+ consumer (Aksoy et al., 2006a). This role of CD38 as an intracellular NADase was proven right when mice lacking CD38 displayed a 30-fold increase in intracellular NAD^+ levels (Aksoy et al., 2006b). This increase is far superior compared to the ~2-fold increases generally observed in most genetic (PARP-1 deletion), pharmacological (NAD^+ precursors) or physiological interventions (fasting, calorie restriction) aimed to enhance NAD^+ content. Given such an increase in NAD^+, it would be expected that CD38 deletion might have a major impact on sirtuin activity. It was therefore reassuring to see how, as happened with PARP-1, CD38 deletion significantly activated SIRT1 and prompted similar clinical phenotypes to those expected for SIRT1 activation, including protection against diet-induced obesity and a robust deacetylation of SIRT1 targets (Aksoy et al., 2006a). Given the nuclear and plasma membrane localization of CD38, it will be interesting to evaluate in the future whether the increase in NAD^+ is homogeneous between compartments and whether all sirtuins are similarly affected.

CONCLUSIONS AND FUTURE PERSPECTIVES

Different strategies aimed to modify the intracellular NAD^+ content have provided correlative evidence that higher NAD^+ bioavailability is matched by higher sirtuin activity. However, this assertion still needs some further work. For example, most studies have focused their attention on the mammalian SIRT1. The mammalian sirtuin family, however, comprises seven distinct members distributed in different cellular compartments. It will be key to elucidate whether the different physiological, pharmacological and genetic manipulations that boost NAD^+ availability lead to an homogenous increase in the activity of all sirtuin enzymes, or whether only a few family members are affected, either due to their different enzymatic kinetics for NAD^+ or to the probable compartmentalization of the modulation in NAD^+ levels. This latter possibility could be highly relevant to design therapeutic strategies aimed to selectively activate specific sirtuin members. It will therefore be crucial to improve the currently available techniques used to evaluate intracellular NAD^+ content and determine sirtuin-specific activity. We also will have to devote efforts to fully understand how the activity of the different sirtuins is affected by other factors, ranging from transcriptional, translational to post-translational processes, and how this interrelates with

effects on NAD^+ kinetics/compartmentalization. A nice example of the complexity of sirtuin biology can be found in the regulation of PGC-1α deacetylation by SIRT1. AMPK-induced PGC-1α phosphorylation facilitates its subsequent deacetylation by SIRT1 (Canto et al., 2009; Jager et al., 2007), indicating that the activity of sirtuins on certain substrates might not only coupled to NAD^+ availability, but also to the accessibility of the target, which is determined by another post-translational modification. Another example can be found in how SIRT1 function is influenced by the ability of different molecular coactivator (e.g. GCN5) of corepressor (e.g. NCoR1 and HDAC3) complexes to interact with SIRT1 or SIRT1 targets (Gerhart-Hines et al., 2007; Picard et al., 2004).

Despite these shortcomings, a steadily increasing amount of evidence indicates that the pharmacological modulation of NAD^+ levels could be a valid strategy to manage metabolic and age-related diseases. PARP inhibitors have been reported to be an attractive tool to enhance oxidative metabolism and promote mitochondrial biogenesis (Bai et al., 2011b). Similarly, the striking ability of NR to enhance yeast lifespan (Belenky et al., 2007) warrants future study to further define the mechanisms through which NR and other NAD precursors might impact on mammalian metabolism. It will also be important to evaluate whether NR might be a valid substitute to avoid the undesirable side effects of other NAD^+ precursors, such as NA and NAM, when used as lipid lowering compounds.

Acknowledgments

The work in the laboratory of the authors was supported by grants of the Ecole Polytechnique Fédérale de Lausanne, Swiss National Science Foundation, NIH (DK59820), the European Research Council Ideas programme (Sirtuins; ERC-2008-AdG23118), and the Velux foundation. The authors thank all the members of the Auwerx lab for inspiring discussions.

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