Exploring the therapeutic space around NAD+

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NAD+ is a central metabolite in the cell. Changes in NAD+ abundance and the activity of NAD+-dependent enzymes, such as the sirtuins, are at the core of metabolic/mitochondrial diseases, such as obesity and diabetes, and of cancer and neurodegeneration. Here, we discuss how maintaining or raising NAD+ levels can improve metabolism and prevent age-related functional decline and associated disease, and how basic scientific discoveries in the NAD+ signaling pathway are being translated to the clinic.

Nicotinamide adenine dinucleotide (NAD) is a metabolite that was first identified over 100 years ago. At that time, Nobel laureate Sir Arthur Harden discovered a molecular fraction involved in yeast fermentation that he called "cozymase." Subsequent work of, among others, three additional Nobel laureates, notably Otto Warburg, led to the identification of NAD+ as an essential cofactor of many biochemical reactions either in its oxidized (NAD^+) or reduced (NADH) form (Berger et al., 2004; Houtkooper et al., 2010a). For instance, NAD+ is used as a cofactor in glucose and fat breakdown, whereas NADH is used as a substrate for the electron transport chain (Fig. 1). NAD+ precursors have been used extensively as dietary supplements: nicotinic acid (NA; also known as niacin) and nicotinamide (NAM) have been long used to treat pellagra, a niacin deficiency (Elvehjem et al., 1937). Niacin is also a commonly used antihyperlipidemic drug. Yet it is only in recent years, after the discovery of the sirtuin protein family, that NAD+ metabolism has emerged as a hot topic for drug development. The key discoveries that led to this increased interest in NAD+ metabolism were the finding that yeast Sir2p was required for the lifespan extension brought about by caloric restriction (Lin et al., 2000), and the identification of sirtuins as NAD+-dependent enzymes (Imai et al., 2000). These two key findings suggested that this class of enzymes could "sense" the metabolic state of the cell, as the NAD+/NADH ratio is largely influenced by the availability and breakdown of nutrients. Because some of the sirtuins in turn regulate the activity of key (transcriptional) metabolic regulators, such as FOXOs, PGC-1α, and p53, the NAD+/sirtuin axis was shown to play a predominant regulatory role in metabolism (Fig. 2 A). Clinical interest was primarily raised by the finding that NAD+/sirtuins promote mitochondrial function, which has made this pathway an attractive target for treating diseases with a mitochondrial contribution, ranging from genetic mitochondrial diseases to cancer and common age-related diseases, including those of the metabolic and nervous systems (Guarente, 2008; Houtkooper et al., 2012). Here, we discuss different means of NAD+ modulation with a particular focus on compounds such as NAD+ precursors, PARP inhibitors, and resveratrol, and describe how these induce mitochondrial function and translate to clinical applications.

NAD+ metabolism—the bench

The biochemistry of NAD+ metabolism. The central role of NAD+ in nutrient breakdown and metabolic sensing requires a delicate balance in its production and utilization. NAD+ levels are controlled by balancing biosynthesis and salvage on one side, and breakdown on the other (Fig. 2 B). NAD+ can be synthesized from the amino acid tryptophan, but its prime precursors include NA and NAM and the more recently identified nicotinamide riboside (NR; Fig. 2 B; Bieganowski and Brenner, 2004). Conversely, the main NAD+ breakdown pathways involves three enzyme classes, i.e., sirtuins, poly(ADP-ribose) polymerases (PARPs), and cyclic ADP-ribose synthases (Fig. 2 B). Although PARPs and cyclic ADP-ribose synthases play a crucial role in NAD+ homeostasis, their function by itself is less relevant for our story, which is why we will not discuss them in detail and refer the reader to a recent review (Houtkooper et al., 2010a).

Modulation of NAD+ levels. The NAD+/sirtuin pathway adapts metabolism in response to environmental (nutrition, exercise) challenges. During energy crises such as caloric restriction, fasting, and exercise, NAD+ levels rise, concomitant with sirtuin activation (Chen et al., 2008; Cantó et al., 2010), while energy overload, such as seen with a high-fat diet, reduces the NAD+/NADH ratio (Kim et al., 2011).

Besides physiological processes, NAD+ levels can be modulated pharmacologically. As a case in point, the polyphenol

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Abbreviations used in this paper: AMPK, AMP-activated protein kinase; NAD, nicotinamide adenine dinucleotide; NR, nicotinamide riboside; PARP, poly(ADP-ribose) polymerase.

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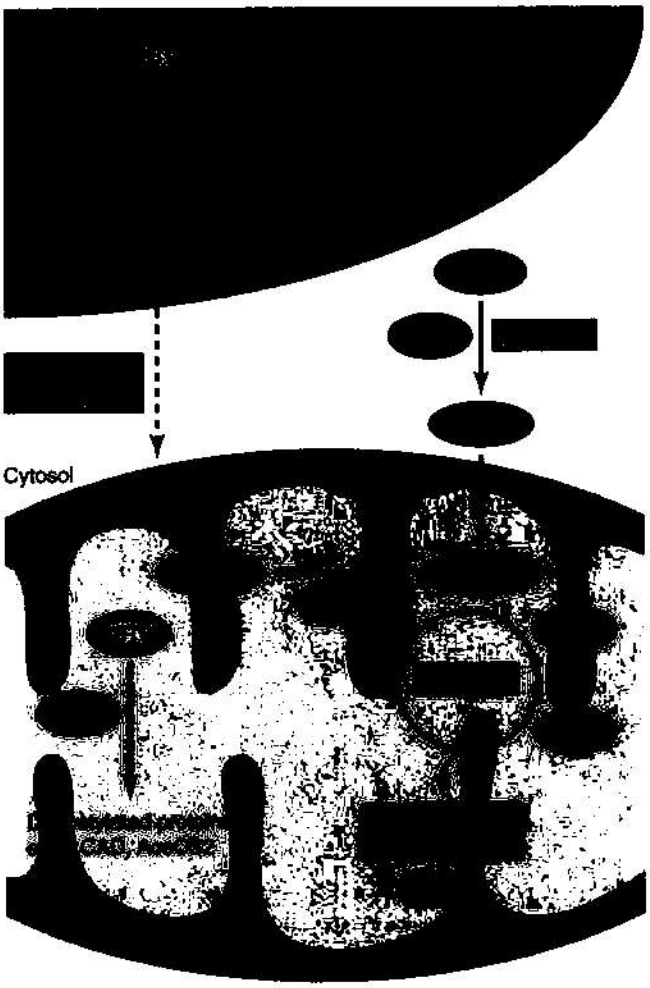

Figure 1. **Diverse metabolic actions of NAD⁺.** Schematic overview of how NAD⁺ and NADH are used as metabolic cofactors, either in more traditional oxidoreductase reactions (green NAD⁺) or in more recently discovered regulatory roles as a cosubstrate for the reactions catalyzed by sirtuins and PARPs (red NAD⁺). Note: reactions shown here are only a small representation of those that involve NAD⁺.

resveratrol, often described as a red wine compound, activates the energy sensor AMP-activated protein kinase (AMPK; Baur et al., 2006; Cantó et al., 2009), which in turn stimulates NAD⁺ (re)synthesis (Fulco et al., 2008; Cantó et al., 2009). Alternatively, supplying precursors or inhibiting NAD⁺ consumers also increases NAD⁺ levels. Supplementation of NA, NR, or NAD⁺ biosynthesis intermediates, like nicotinamide mononucleotide (NMN), increases NAD⁺ levels in cultured cells or in tissues of mice receiving the compounds (Yoshino et al., 2011; Cantó et al., 2012). Similarly, treatment with either inhibitors of PARPs (Bai et al., 2011b) or CD38 (Barbosa et al., 2007), both enzymes that consume NAD⁺, will induce NAD⁺ levels and results in the activation of the sirtuins. Importantly from a therapeutic perspective, because NR and probably also the other precursors can be metabolized both in the nucleus and in mitochondria, NR supplementation leads to accumulation of nuclear and mitochondrial NAD⁺, and subsequent activation of both the nuclear SIRT1 and the mitochondrial SIRT3 (Cantó et al., 2012). On the other hand, the NAD⁺ consumer PARP1 is confined to the nucleus. This implies that increased NAD⁺ levels in tissues of *Parp1* knockout mice are accompanied by nuclear SIRT1, but not mitochondrial SIRT3, activation (Bai et al., 2011b). Future work will have to address whether PARP inhibitors also activate the other nuclear sirtuins, SIRT6 and SIRT7, and how inhibitors of the ectoenzyme CD38 modulate subcellular NAD⁺ levels and sirtuin activity.

Sirtuin activation and mitochondrial metabolism. The yeast sirtuin enzyme Sir2p was described to prevent genome instability in yeast, thereby acting as an anti-aging factor (Haigis and Sinclair, 2010; Houtkooper et al., 2012). In mammals, the sirtuin family consists of seven members, SIRT1–7, with distinct enzymatic activities and subcellular localization (Houtkooper et al., 2012). As discussed earlier, sirtuins function mainly as deacetylases and are optimally placed to translate changes in nutritional state to metabolic adaptations. SIRT1 is the best-characterized sirtuin and is implicated in many nonmetabolic processes as well. The prime metabolic target for SIRT1 is PGC-1α, a cofactor that regulates mitochondrial biogenesis and function (Fig. 2 A; Lin et al., 2005). From the other SIRT1 deacetylation targets, the forkhead transcription factor FOXO1 is worth mentioning in this context as it modulates mitochondrial fatty acid metabolism and protects against oxidative stress (van der Horst and Burgering, 2007). The key mitochondrial sirtuin, SIRT3, targets several proteins involved in fatty acid metabolism and ketogenesis, and antioxidant enzymes (Fig. 2 A; Houtkooper et al., 2012), although its real physiological role in metabolic control is still debated (Hirschey et al., 2010; Fernandez-Marcos et al., 2012). Through this multitude of sirtuin targets, NAD⁺ has a profound effect on mitochondrial function in general, although future work will need to clarify how these pleiotropic actions are controlled by specific sirtuins.

Clinical application of NAD⁺ metabolism—the bedside

With the central position of NAD⁺ in cellular metabolism in mind, the question is whether increasing its level could indeed be clinically beneficial, similar to the effects of caloric restriction. The key to answering this question is whether NAD⁺ is a limiting factor for the enzymes that require NAD⁺ as a cosubstrate. In support of this premise is the fact that high-dose vitamin and micronutrient therapy have been known to yield pleiotropic beneficial effects on multiple metabolic pathways (Ames, 2006). Boosting NAD⁺ levels may hence have similar favorable effects on pathways that require NAD⁺ as a cosubstrate.

Hyperlipidemia. For a long time, patients with hyperlipidemia have been treated with niacin (Altschul et al., 1955), often in combination with statins to lower cholesterol biosynthesis. Although the G protein–coupled receptor GPR109A was linked to the effects of niacin (Tunaru et al., 2003), an increase in NAD⁺ could also contribute, especially considering the central role of sirtuin enzymes in lipid metabolism (Schug and Li, 2011; Houtkooper et al., 2012). Arguing in favor of the latter hypothesis is the fact that high doses of niacin (grams/day) are required for lipid lowering, while the EC_{50} for GPR109A (i.e., concentration to activate 50% of the receptor; ~250 nmol/L)