Review

CellPress

*Special Focus – Metabolism*

# $NAD^+$ and sirtuins in aging and disease

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**Nicotinamide adenine dinucleotide ($NAD^+$) is a classical coenzyme mediating many redox reactions. $NAD^+$ also plays an important role in the regulation of $NAD^+$-consuming enzymes, including sirtuins, poly-ADP-ribose polymerases (PARPs), and CD38/157 ectoenzymes. $NAD^+$ biosynthesis, particularly mediated by nicotinamide phosphoribosyltransferase (NAMPT), and SIRT1 function together to regulate metabolism and circadian rhythm. $NAD^+$ levels decline during the aging process and may be an Achilles' heel, causing defects in nuclear and mitochondrial functions and resulting in many age-associated pathologies. Restoring $NAD^+$ by supplementing $NAD^+$ intermediates can dramatically ameliorate these age-associated functional defects, counteracting many diseases of aging, including neurodegenerative diseases. Thus, the combination of sirtuin activation and $NAD^+$ intermediate supplementation may be an effective antiaging intervention, providing hope to aging societies worldwide.**

## $NAD^+$ as an essential compound for many enzymatic processes

$NAD^+$ was discovered more than a century ago by Sir Arthur Harden, as a low molecular weight substance present in a boiled yeast extract that could stimulate fermentation and alcohol production *in vitro* [1]. Subsequent studies over the next several decades determined that the structure of $NAD^+$ comprised two covalently joined mononucleotides [nicotinamide mononucleotide (NMN) and AMP] and identified the keystone function of $NAD^+$ and NADH as enzyme cofactors mediating hydrogen transfer in oxidative or reductive metabolic reactions [1].

For an extended period, $NAD^+$ thus appeared in biochemistry textbooks with the sole function of a cofactor of enzymes serving metabolic pathways in cells. More recently, $NAD^+$ has been associated with biochemical reactions other than hydrogen transfer, serving as a cosubstrate for bacterial DNA ligase [2], PARP [3], CD38/157 ectoenzymes [4], and class III $NAD^+$-dependent deacylases or sirtuins [5]. In all of these newer examples, $NAD^+$ is cleaved at the glycosidic bond between nicotinamide and ADP-ribose (Figure 1; described in detail below). For the ligase, ADP-ribose is transferred to the 5' hydroxyl of DNA to be ligated. For PARP, ADP-ribose is serially transferred to arginine side chains in itself, histones, and other proteins at sites of DNA damage. For CD38/157, $NAD^+$ is provided through the connexin 43 hemichannels and hydrolyzed extracellularly. These enzymes also generate cADP-ribose (cADPR), a strong $Ca^{2+}$ inducer. Lastly, for sirtuins, $NAD^+$ cleavage catalyzes the removal of acetyl or acyl groups from lysines of sirtuin substrate proteins accompanied by their transfer to ADP-ribose.

Much excitement arose from the idea that sirtuins regulate health and lifespan in many different organisms in accord with diet. In particular, it was shown that $NAD^+$ and NADH could vary with the availability of dietary energy and nutrients. For example, an increase in $NAD^+$ (or decrease in NADH) was proposed to mediate the extension of life and health span by dietary restriction (DR) [6]. This study challenged the dogma arising from earlier studies, which found that $NAD^+$ was present in excess of NADH in cells and did not vary much with diet [7]. Reciprocally, many recent studies have provided evidence that defects in maintaining $NAD^+$ levels and the accompanying decline in activity of sirtuins may help drive normal aging [8,9]. These latter studies are additionally exciting because they also demonstrate that $NAD^+$ deficiency and associated pathologies may be normalized by supplementation with $NAD^+$ precursors and intermediates. This review expands on this new framework, considering aging and diseases, and discusses the emergence of approaches to counter effects of aging by small molecules that can rescue defects in $NAD^+$ and sirtuin activity.

## $NAD^+$ plays a key role in regulating metabolism and circadian rhythm

The canonical role of $NAD^+$, mentioned above, is to facilitate hydrogen transfer in key metabolic pathways (Figure 1A). For example, $NAD^+$ is converted to NADH in the glyceraldehyde 3-phosphate dehydrogenase step of glycolysis, a pathway in which glucose is converted to pyruvate. Conversion of $NAD^+$ to NADH is also important in mitochondrial metabolism. In that compartment, $NAD^+$ is converted to NADH in four steps of the mitochondrial tricarboxylic acid (TCA) cycle, in which acetyl-coenzyme A (CoA) is oxidized to carbon dioxide. $NAD^+$ is also converted to NADH during the oxidation of fatty acids and amino acids in mitochondria. In these mitochondrial pathways, the NADH generated is an electron donor for oxidative phosphorylation and ATP synthesis.

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*Keywords:* $NAD^+$; sirtuins; poly-ADP-ribose polymerases; nicotinamide phosphoribosyltransferase; nicotinamide mononucleotide; nicotinamide riboside.

0962-8924/
 http://dx.doi.org/10.1016/j.tcb.2014.04.002

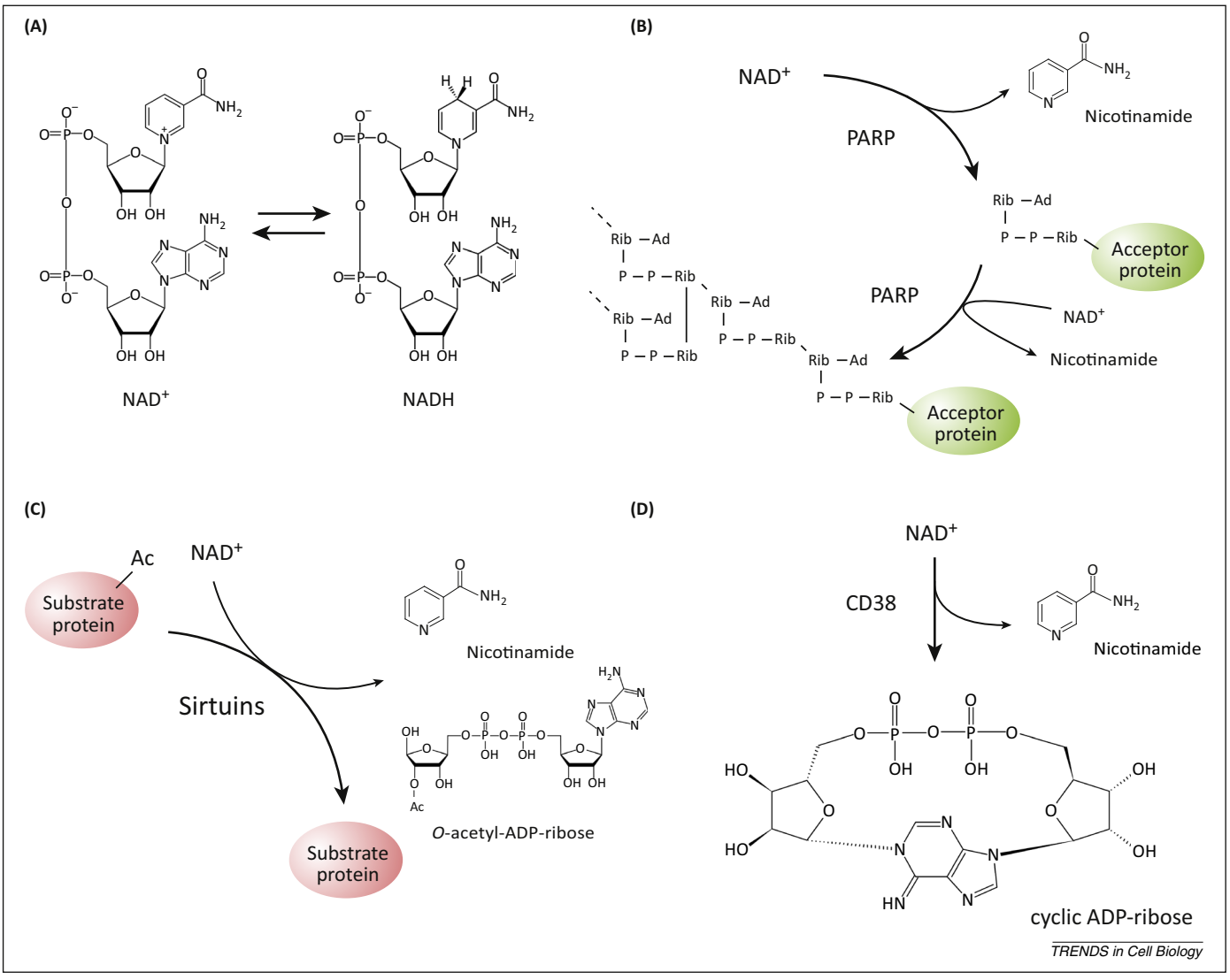


**Figure 1**. Various uses of $NAD^+$ for canonical redox and $NAD^+$-consuming enzymatic reactions. Whereas $NAD^+$ is converted to NADH by many metabolic enzymes **(A)**, it is also used as a cosubstrate for $NAD^+$-consuming enzymes such as poly-ADP-ribose polymerases (PARPs) **(B)**, sirtuins **(C)**, and CD38/157 ectoenzymes **(D)**.

In addition to these canonical uses of $NAD^+$ and NADH, PARPs transfer ADP-ribose from $NAD^+$ to itself, histones, and other proteins at sites of DNA damage to facilitate repair and maintenance of genomic integrity (Figure 1B). Damaged DNA recruits PARP and activates its poly-ADP-ribosylation activity *in situ*. Thus, acute DNA damage, for example by ionizing radiation, can trigger a sudden depletion of $NAD^+$ due to PARP activation. PARP inhibitors are in clinical trials as anticancer agents [10], because they can sensitize tumor cells to apoptotic killing by genotoxic agents through the prevention of DNA repair.

Sirtuins are $NAD^+$-dependent deacylases that play key roles in responding to nutritional and environmental perturbations such as fasting, DR, DNA damage, and oxidative stress (Figure 1C). In general, their activation triggers nuclear transcriptional programs that enhance metabolic efficiency and upregulate mitochondrial oxidative metabolism and the accompanying resistance to oxidative stress [11]. Sirtuins foster this resistance by increasing antioxidant pathways [e.g., superoxide dismutase 2 (SOD2) and isocitrate dehydrogenase 2 (IDH2) in mitochondria] and by facilitating DNA damage repair through deacetylation or ADP-ribosylation of repair proteins [12]. Accordingly, many studies have shown that sirtuins promote longevity in yeast, worms, flies, and mice and can mitigate many diseases of aging in murine models, such as type 2 diabetes, cancer, cardiovascular diseases, neurodegenerative diseases, and proinflammatory diseases [11,13,14]. Although a challenge was raised to the proposed conserved role of sirtuins in aging/longevity control [15] (Box 1), many recent studies have upheld the original claims [16–23].

Among the many ways sirtuins influence metabolism is by regulating the circadian clock machinery. SIRT1, the most studied member of mammalian sirtuins, deacetylates central clock components in the liver [24,25] and amplifies the expression of the circadian transcription factors BMAL and CLOCK in the suprachiasmatic nucleus (SCN) of the hypothalamus via deacetylation of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) [26]. In the latter case, loss of SIRT1 function occurs with aging, which results in damped levels of the clock components and deterioration of central circadian control. Defects in central circadian control have been associated