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Review

Mitochondrial Sirtuins and Molecular Mechanisms of Aging

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Advancing age is the major risk factor for the development of chronic diseases and is accompanied by changes in metabolic processes and mitochondrial dysfunction. Mitochondrial sirtuins (SIRT3–5) are part of the sirtuin family of NAD$^+$-dependent deacylases and ADP-ribosyl transferases. The dependence on NAD$^+$ links sirtuin enzymatic activity to the metabolic state of the cell, poising them as stress sensors. Recent insights have revealed that SIRT3–5 orchestrate stress responses through coordinated regulation of substrate clusters rather than of a few key metabolic enzymes. Additionally, mitochondrial sirtuin function has been implicated in the protection against age-related pathologies, including neurodegeneration, cardiopathologies, and insulin resistance. In this review, we highlight the molecular targets of SIRT3–5 and discuss their involvement in aging and age-related pathologies.

Decreased Mitochondrial Function in Aging

Aging is accompanied by the degeneration of multiple organ systems, leading to mortality. This decline in organ systems is associated with a loss of cellular homeostasis in fundamental pathways, such as genome fidelity, proteostasis, and nutrient sensing [1]. Intriguingly, mitochondria are at the center of multiple pathways in homeostasis due to their central role in bioenergetics, catabolic and anabolic metabolism, generation of reactive oxygen species (ROS), apoptosis, and signal transduction. These organelles are dynamic and reprogram metabolism in response to cellular stress. Unsurprisingly, mitochondrial dysfunction has been linked to numerous aspects of aging, including decreased activity of metabolic enzymes, impaired respiratory capacity, and increased oxidative damage [2]. Recent advances have demonstrated that decreased NAD$^+$ contributes to cellular and mitochondrial decline during aging [3]. NAD$^+$ functions as a cofactor for numerous metabolic enzymes, and is a co-substrate for the **sirtuin family of deacylases** (see Glossary) [3–5]. In this review, we discuss recent advances in the identification of mitochondrial sirtuin (SIRT3–5) substrates and describe the mitochondrial programs that are regulated by SIRT3–5 in response to mitochondrial stress. Finally, we highlight the most recent work connecting SIRT3–5 to aging and age-related diseases.

Mitochondrial Sirtuins are NAD$^+$-Dependent Enzymes

Sirtuins are NAD$^+$-dependent enzymes conserved from bacteria to humans [6]. Mammals contain seven sirtuin enzymes (SIRT1–7). While the catalytic core domain contains amino acid

Trends

- Mitochondrial sirtuins SIRT3, SIRT4, and SIRT5 are NAD$^+$-dependent deacetylases, deacylases, and ADP-ribosyl transferases. Their dependence on NAD$^+$ directly links their enzymatic activity to the metabolic state of the cell.
- In mammalian tissues, mitochondrial sirtuin expression and/or activity may decline with age and contributes to mitochondrial dysfunction, a major hallmark of aging. Additionally, loss-of-function studies in genetic mouse models have linked decreased sirtuin function to the development of age-related diseases, including neurodegeneration, insulin resistance, and heart disease.
- Supplementation of NAD$^+$ improves healthspan and restores mitochondrial homeostasis in model systems.
- Recent insights have revealed that mitochondrial sirtuins affect many facets

residues invariant throughout evolution, the N- and C-terminal regions are structurally divergent and contribute to differences in subcellular localization, enzymatic activity, and substrate specificity [7]. Three sirtuins (SIRT3, SIRT4, and SIRT5) localize to the mitochondrial matrix, positioning these enzymes within the metabolic hub of the cell. SIRT3–5 contain an N-terminal mitochondrial signal sequence that dictates sirtuin localization to these organelles; here, they coordinate numerous facets of mitochondrial biology with important implications for aging and disease [4,7]. For instance, SIRT3 boosts mitochondrial oxidative metabolism in response to nutrient stress and membrane depolarization [8,9]. Consequently, loss of SIRT3 has been mechanistically linked to decreased heart function and neurodegeneration [10,11].

Elucidating the enzymatic activity of sirtuins is a dynamic area of investigation. Initially, sirtuins were identified as NAD^+-dependent deacetylases or mono ADP-ribosyltransferases. Recent work has revealed that sirtuins catalyze a range of NAD^+-dependent reactions, including deacetylation, deacylation, and ADP ribosylation [3–5]. Hence, sirtuins are often classified as 'deacylases' to account for the notion that they are able to remove a variety of long acyl moieties from substrates including succinyl, malonyl, and lipoyl groups, as well as bulkier palmitoyl and myristoyl modifications [12]. During catalysis, sirtuins use NAD^+ to remove the acyl group from lysine to form 2′-*O*-acyl-ADP-ribose and nicotinamide (Figure 1A). Additionally, some sirtuins, including SIRT4 and SIRT6, display ADP-ribosyl transferase activity, used to transfer ADP-ribose from NAD^+ to the substrate, thus yielding nicotinamide as a product (Figure 1B). The dependence on NAD^+ as a co-substrate positions sirtuins as critical sensors of the metabolic and redox states of a cell [4].

The biology of NAD^+ is among the most dynamic areas of aging research. The ratio between NAD^+ and its reduced counterpart NADH is intricately tied to cellular and mitochondrial metabolism, and NAD^+:NADH ratios and levels are affected by dozens of cellular reactions. In mitochondria, NADH is generated from glycolysis and the **tricarboxylic (TCA) cycle**. NAD^+ can be regenerated through numerous reactions, including oxidation of NADH by **complex I**. The donated electrons feeding into the **electron transport chain** (ETC) ultimately contribute to the build-up of a proton gradient that is used by ATP synthase to produce ATP. In addition, NAD^+ can be generated through *de novo* synthesis, salvage pathways, or oxidation of NADH by lactate dehydrogenase (LDH) in glycolytic cells.

NAD^+ levels decline during aging, and there is compelling evidence that genetic manipulation of NAD^+ biosynthesis or supplementation with NAD^+ precursors can extend lifespan in model organisms spanning yeast, worms, and mice [13–18]. While mitochondria appear to be protected against stress-induced decreases in NAD^+, they can only retain optimal NAD^+ levels for a short period of time [19–21]. Given that mitochondrial sirtuin activity may decline in conditions of decreased NAD^+, for instance during stress and aging, the inability to regulate appropriate responses to acute mitochondrial stress can severely impair cellular homeostasis [22]. Indeed, several studies have reported that restoration of NAD^+ levels during stress or aging may directly activate mitochondrial sirtuins to restore homeostasis [14,21]. Due to the scope of this review, we do not focus in-depth on the benefits of NAD^+ restoration and lifespan improvement, unless directly linked to the discussion of mitochondrial sirtuins. For more in-depth reviews on NAD^+ and aging, see [3,22,23].

Mitochondrial Sirtuins Regulate Protein Networks to Orchestrate the Stress Response

While initial studies focused on identifying individual substrates for mitochondrial sirtuins, recent work has focused on elucidating the networks associated with sirtuins. An emerging idea from these studies is that sirtuins do not regulate the activity of a few key substrates, but rather, regulate functional clusters of targets to orchestrate a coordinated, physiological response

of mitochondrial biology through the regulation of vast networks of metabolic and non-metabolic enzymes, thus ensuring that mitochondrial homeostasis is achieved during stress conditions.

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