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Review Article

Exploring the electrostatic repulsion model in the role of Sirt3 in directing MnSOD acetylation status and enzymatic activity

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ABSTRACT

Mitochondrial oxidative metabolism is the major site of ATP production as well as a significant source of reactive oxygen species (ROS) that can cause damage to critical biomolecules. It is well known that mitochondrial enzymes that scavenge ROS are targeted by stress responsive proteins to maintain the fidelity of mitochondrial function. Manganese superoxide dismutase (MnSOD) is a primary mitochondrial ROS scavenging enzyme, and in 1983 Irwin Fridovich proposed an elegant chemical mechanism/model whereby acetylation directs MnSOD enzymatic activity. He christened it the "electrostatic repulsion model." However, the biochemical and genetic mechanism(s) determining how acetylation directs activity and the reasons behind the evolutionarily conserved need for several layers of transcriptional and posttranslational MnSOD regulation remain unknown. In this regard, we and others have shown that MnSOD is regulated, at least in part, by the deacetylation of specific conserved lysines in a reaction catalyzed by the mitochondrial sirtuin, Sirt3. We speculate that the regulation of MnSOD activity by lysine acetylation via an electrostatic repulsion mechanism is a conserved and critical aspect of MnSOD regulation necessary to maintain mitochondrial homeostasis.

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Acetylation in the regulation of mitochondrial proteins

Eukaryotic cells contain posttranslational adaptive signaling pathways for responding to oxidative stress that induce the enzymatic activity of ROS scavenging proteins to maintain metabolic homeostatic poise. These signaling pathways permit the cell to exploit and adapt to environmental conditions, thereby minimizing the potentially deleterious effects of oxidative metabolism. Over the last few years, several proteomic studies have suggested that the

Abbreviations: s: ROS, reactive oxygen species; CR, caloric restriction; MnSOD, manganese superoxide dismutase; CuZnSOD, copper zinc superoxide dismutase; EcSOD, extracellular superoxide dismutase; $O_2^{\bullet -}$, superoxide; H_2O_2, hydrogen peroxide; $HO^{\bullet}$, hydroxyl radical; Sirt1–7, Sirtuin-1–7; RIRR, ROS-induced ROS release.

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cellular acetylome is roughly two-thirds the size of the kinome, suggesting that protein acetylation may play a key role in the regulation of signaling cascades [1–3]. Acetylation of lysine residues neutralizes positive charges on proteins, subsequently altering their three-dimensional structure as well as changing enzymatic function [4,5]. In this regard, lysine acetylation has emerged as a critical posttranslational modification employed to modify mitochondrial proteins as well as regulate protein catalytic activity [3,6]. The idea that the mitochondrial acetylome directs cellular metabolism is based on several proteomic surveys identifying a high number of acetylated proteins in the mitochondria that appear to direct metabolism [1,3]. These studies clearly suggested that the acetylome, via changes in lysine acetylation, regulates a wide spectrum of metabolic pathways employed to direct energy production [1,3,7] in a carbon source-dependent fashion [8].

A key observation regarding mitochondrial biology is that mice placed on a caloric restriction (CR) diet display a very significant change in both global and mitochondrial protein acetylation [9,10]. For example, large-scale mass spectrometry screening pre- and post-CR identified well over 100 reversibly acetylated lysines in 72 mitochondrial proteins from a wide variety of metabolic pathways [11]. Interestingly, CR or other forms of nutrient stress have been investigated for many years and have been shown to prevent or reverse age-related changes in multiple murine phenotypes, including insulin resistance and neurodegenerative diseases, and to decrease the incidence of carcinogenesis in tumor permissive mice. Thus, over the last few years there has been mounting evidence that various forms of nutrient stress, such as exhaustive exercise, fasting, or CR, can profoundly alter mitochondrial physiology and pathophysiology associated with aging as well as age-related illnesses [12]. Based on these results, it seems reasonable to suggest that acetylation of mitochondrial proteins may play a role in maintaining and regulating mitochondrial metabolism and function.

Importance of metabolic homeostasis

For many years it has been known that the maintenance of metabolic homeostasis in mammalian cells requires a series of biochemical oxidation/reduction (redox) reactions involving protein catalysts [13,14]. Mammalian cellular processes primarily derive energy from the tightly controlled regulation of oxidative metabolism, which biochemically oxidizes substrates (i.e., carbohydrates, fats, and amino acids) [15,16]. The oxidation of substrates is used to obtain reducing equivalents (electrons) necessary for mitochondrial electron transport-mediated oxidative phosphorylation to produce ATP, with O_2 acting as the terminal electron acceptor [17–19]. Furthermore, reducing equivalents are also involved in many cellular processes including gene replication and transcription and protein synthesis, as well as maintaining the cellular reducing environment [14,20,21]. However, oxidative metabolism is also capable of generating ROS [e.g., superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$)] as by-products [22,23] that can act as both signaling molecules and damaging agents. Through a series of biochemical oxidation/reduction (redox) reaction processes, $O_2^{\bullet-}$ is dismuted to hydrogen peroxide, H_2O_2, either spontaneously or enzymatically in the presence of superoxide dismutase enzymes [i.e., copper zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), extracellular superoxide dismutase (EcSOD)] [24–26].

Steady-state levels of ROS are a function of both production via aerobic respiration and removal by antioxidant scavenging enzymes [22,27]. Under normal circumstances, it is estimated that 1% or less of total O_2 consumption results in $O_2^{\bullet-}$ and H_2O_2 formation through mitochondrial electron transport chains [28,29]. This low level of ROS is essential for many normal cellular processes including cell signaling, cell adhesion, cellular immune response, apoptosis, and cell survival [30–33]. In addition, antioxidant capacity can shift metabolism away from pathways that produce ROS as well as prevent ROS reactions with critical biomolecules. However, under certain circumstances such as exposure to exogenous stress or the aging process, metabolic prooxidant production can exceed antioxidant capacity, leading to oxidative stress [22,34]. The excess ROS can then react with a broad range of biomolecules including lipids, proteins, and DNA to form other radicals or cytotoxic by-products. For instance, lipid peroxidation can lead to the oxidative degradation of lipids to form reactive and cytotoxic products [31]. Since this process proceeds by a free radical chain reaction mechanism, lipid peroxidation not only affects polyunsaturated fatty acids, but it could also amplify the number of free radicals as well as produce diffusible cytotoxic by-products [15]. It is widely thought that maintaining the balance of metabolic homeostasis is critical to cell fate and that increased ROS production by abnormal redox metabolism leads to disrupted regulation of gene expression and aberrant protein activities that could contribute to cell injury, mutagenesis, senescence, teratogenesis, carcinogenesis, and cell death [35–37].

MnSOD in the maintenance of cellular metabolic homeostasis

The proper regulation of mitochondrial function as well as maintenance of mitochondrial oxidative metabolism is critical for minimizing the accumulation of potentially damaging ROS [38]. In addition, the mitochondria represent the primary site of superoxide production through the process of respiration via the electron transport chain [39,40]. While low levels of superoxide and/or other ROS are easily tolerated by the cell, abnormally high levels of ROS from any number of possible sources induce oxidative stress and can damage cells [25]. As such, the mitochondria contain specific processes to scavenge and remove ROS in order to maintain homeostasis.

In humans there are three forms of SOD: cytosolic CuZnSOD, mitochondrial MnSOD, and EcSOD [37]. Among them, MnSOD is the major enzymatic superoxide scavenger inside the mitochondrial matrix and is considered one of the most critical ROS scavenging/detoxification enzymes in the cell, one that is absolutely necessary for an organism to survive and produce ATP in an oxygen-rich environment [41,42]. Human MnSOD is a tetrameric enzyme complex that is made up of four identical subunits each harboring a Mn^{3+} atom (Fig. 1A) that converts two $O_2^{\bullet-}$ molecules to one O_2 and one H_2O_2 through a ping-pong catalytic mechanism characterized by redox transitions of Mn^{3+} to Mn^{2+} and back to Mn^{3+} [24,43,44]. Since the production of $O_2^{\bullet-}$ is a function of the rate of one-electron reductions of O_2 within the mitochondria, it seems reasonable to propose that the mitochondria would contain watchdog or sensing proteins to regulate oxidative metabolic processes, including the activity of detoxification enzymes such as MnSOD, to maintain metabolic homeostasis as well as prevent cellular damage during oxidative metabolism.

Sirt3 deacetylates and regulates MnSOD enzymatic activity

As suggested above, lysine acetylation has recently emerged as an important posttranslational modification employed to regulate mitochondrial proteins [45–47]. Thus, lysine acetylation, regulated by either acetyl transferases or deacetylases, may play an important role in metabolic homeostasis by coordinating mitochondrial oxidative metabolism and ROS levels to match ATP production with intracellular energy requirements. In this regard,