ROS

REVIEW ARTICLES

# Reactive Oxygen Species Regulate Myocardial Mitochondria through Post-Translational Modification

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**ABSTRACT |** Post-translational modifications (PTMs) generally refer to covalent and enzymatic modifications of proteins during or after protein biosynthesis, as well as during cleavage or degradation of proteins. These modification processes govern not only protein homeostasis but also new cellular protein functions. As an essential part of complex cellular signal sensing and transduction networks, PTMs respond to various reactive oxygen species (ROS) and contribute to multi-organ injuries although the precise underlying mechanisms of action remain poorly understood. Up to date, a number of PTMs have been postulated to participate in the regulation of mitochondrial function through free radical species and other second messengers. Mitochondrion is believed to serve as a main ROS generator, and at the same time, also an ROS receptor. Modification of mitochondrial proteins may result in detrimental biological consequences through oxidative injury and mitochondrial dysfunction. In this mini-review we will recapitulate some aspects of PTMs in the control of mitochondrial integrity in the heart. The major forms of PTM related to mitochondrial regulation will be discussed here, which include phosphorylation, ubiquitination, acetylation, and oxidative and nitrosative modification.

**KEYWORDS |** Mitochondria; Oxidative stress; Post-translational modification

**ABBREVIATIONS |** CoQ10, coenzyme Q10; ETC, electron transport chain; NO, nitric oxide; OXPHOS, oxidative phosphorylation; Ox-PTM, oxidative post-translational modification; PDH, pyruvate dehydrogenase; PTM, Post-translational modification; RNS, reactive nitrogen species; ROS, reactive oxygen species; SNO, S-nitrosylation; STAT3, signal transducer and activator of transcription 3; Ub, ubiquitin

**CONTENTS**

## 1. INTRODUCTION

Heart diseases impose a major health threat with a rather complex etiology. A number of mechanisms have been postulated and widely accepted for the onset and progression of heart diseases including genetic and epigenetic alterations in response to various environmental cues [1–3]. Although recent advances in genetics have greatly enriched our understanding of disease mechanisms [4, 5], various factors beyond genetic coding or control, such as post-translational modifications (PTMs) of essential proteins, may also contribute to the cardiac homeostasis and therefore the etiology of heart diseases [6, 7]. PTMs of proteomes are believed to provide the pieces needed to complete the puzzle between unaltered gene levels and changes in metabolism, morphology, and function for the heart [7]. In particular, PTMs have been demonstrated to link regulation of myocardial metabolic or functional changes with energy protein enzymatic activity (e.g., mitochondrial oxidative capacity) [7]. Mitochondria are known as the dynamic organelles for generation of cardiac fuel (ATP) and as the key regulators for main cellular survival function including autophagy, apoptosis, intracellular calcium homeostasis, and lipid metabolism [8]. Many of these cellular machineries help to maintain mitochondrial integrity and function via mitochondrial quality control in various cardiac stress conditions [9]. Mitochondrial variations and uncoupling can function to promote the production of reactive oxygen species (ROS) and thus further exacerbate cardiac pathologies, or trigger pivotal signaling components for development of cardioprotection [10].

Regulation of post-translational protein modifications is paramount to cardiovascular development, homeostasis, and stress response [7]. In general, such protein regulations can be categorized into two major groups: amino acid modification and group attachment [11]. PTMs usually refer to an addition of small chemical moieties to amino acid residues without interfering with DNA coding sequence. These processes can be shown as phosphorylation, ubiquitination, acetylation, oxidative/nitrosative modifications, hydroxylation, and sulfidation [12]. While these PTMs of essential functional proteins may lead to specific cellular and biological events in the heart and other organs, one main challenge for scientists is how to precisely define the direct functional implications of these modifications [13]. Defining the outcome of modification of a given protein can be challenging as pan protein levels, degree of modification, localization, and activity of the functional component of the protein may contribute to the final pathophysiological outcomes, letting alone the existence of off-target modification for other proteins. More recent evidence has depicted that environmental factors such as nutrients and levels of ROS may help to govern the on/off switch of PTMs for various downstream signal transduction pathways [7].

Ample clinical and experimental evidence has demonstrated the unique role for ROS-triggered modifications of functional proteins as the main risk factors for oxidative damage in pathological cardiovascular processes [7, 14]. However, the ROS-governed modification theory has been challenged as merely mitigating existing ROS by antioxidants is ineffective to decrease mortality of heart failure [15, 16]. Among various antioxidants with somewhat dismal efficacy, coenzyme Q10 (CoQ10) has shown some clinical promises in the therapeutics of cardiovascular diseases [17, 18]. CoQ10, an endogenously synthesized and dietary lipid-soluble cofactor, acts to transfer electrons from complexes I and II to complex III in the mitochondrial inner membrane, thus enabling CoQ10 to function as a membrane antioxidant and benefit congestive heart failure [17]. One of the hypotheses for the lack of success for antioxidants in heart diseases is possibly related to the inability of "regular" antioxidants to scavenge ROS present within mitochondria. To this end, more effective therapeutic strategies are needed to drastically improve the capacity of mitochondrial quality control in an effort to maintain cardiac function [19]. It has been well established that defective or aged mitochondria can have severe consequences by releasing ROS and cytochrome c into cytosol, inducing apoptosis of cardiomyocytes [20]. Mitochondrial quality control is succinctly controlled by a wide array of factors ranging from mitochondrial dynamics to mitochondrial biogenesis and degradation at various points throughout the life course. These procedures may be grouped under the "mitochondrial biogenesis" umbrella although such dynamic chang-

es are not restricted to newborn mitochondria. In fact, clearance of impaired mitochondrial fragments into the cytosol is well executed through the process of mitophagy [21]. Coordination of mitochondria dynamics in an effort to regulate ROS levels requires not only the mitochondrial outer membrane but also attachment of proteins formed in the cytoplasm [22]. In pathological conditions, intra- or extracellular ROS may regulate mitochondrial ion channels/transporters by way of redox-dependent PTMs in a vicious cycle. These redox-sensitive PTMs may be greatly amplified by mitochondrial ROS production, causing another surge of ROS release from the neighboring mitochondria, and ultimately imposing more PTMs and stress in the mitochondria [23]. Nevertheless, little is known about these mitochondrial protein modifications, for instance, the mitochondrial quality control by ubiquitination or other PTMs. In the following sections, we will briefly review and discuss the critical role of ROS and PTMs in regulating mitochondrial quality control in the pathophysiological process of cardiac injury.

## 2. PHOSPHORYLATION

Phosphorylation is perhaps one of the most common and well-studied posttranslational modifications in mammalians [24]. Phosphorylation involves the addition of a reversible bond of a phosphate ester onto given amino acid residues, including serine (Ser), threonine (Thr), and tyrosine (Tyr). Recent evidence revealed a new link between phosphorylation status and dynamic control in the mitochondrial proteome [13]. Mitochondria are well-known as myocardial ‘‘powerhouse’’ organelles for ATP production. Meanwhile, mitochondria also serve as important regulators for multiple redox signaling pathways as majority of mitochondrial ATP is produced by metabolism of fatty acids and oxidative phosphorylation (OXPHOS) via mitochondrial electron transport chain (ETC) [25]. A small portion of electrons escapes from the ETC to reduce molecular oxygen during OXPHOS, generating superoxide anion ($O_2^{\bullet -}$) continuously, and $O_2^{\bullet -}$ serves as the primary oxygen free radical in mitochondria [23]. OXPHOS defects in phosphorylation have been considered as a main trigger for cardiomyopathy and heart failure due to uncontrollable ROS production, and complications of mitochondrial encephalopathy, neuropathy, and myopathy [26]. Although lipids may serve as a main dynamic pool for fatty acids and mitochondrial fuels, they may also function as uncouplers and inhibitors of oxidative phosphorylation [27]. A theory of lipid utilization by mitochondria has been put forward, which helps to maintain ROS within levels compatible with signaling while producing robust and reliable energy supply at the same time [27].

Other than mitochondrial oxidative phosphorylation, other phosphorylation processes also contribute to ROS production and change of mitochondrial function. For example, phosphorylation status and pan-protein levels of signal transducer and activator of transcription 3 (STAT3), a transcriptional molecule, may be dynamic and regulate mitochondrial function. STAT3 is phosphorylated at various residues with functional consequences to protect against oxidative stress [28, 29]. Moreover, evidence from superoxide dismutase 2 (SOD2)-transgenic mice confirmed reduced superoxide production and repressed pyruvate dehydrogenase (PDH) activity and mitochondrial ETC activity in diabetes mellitus. PDH activity, or its phosphorylation, may prevent mitochondrial pyruvate uptake therefore interrupting energy metabolism and promoting ROS production [30]. Elevated PDH phosphorylation was observed in diabetic hearts [31, 32], which may greatly contribute to the overt ROS production and mitochondrial damage along with cardiomyopathy in diabetic hearts [33, 34].

## 3. UBIQUITINATION

Ubiquitin (Ub), a conserved 76-amino acid globular protein, participates in protein degradation, a process known as ubiquitination essential for mitochondrial quality control. Ubiquitination and Ub receptor proteins (e.g., p62 and ubiquilins) are considered key factors to target misfolded proteins for quality control in a varieties of entities, including the proteasome, lysosomes, and mitophagy to remove defective mitochondria [35]. Ubiquitination process involves a family of Ub-conjugating proteins [36]. An early step for ubiquitination is the ATP-dependent activation of free Ub to form a thiol-ester tie between the protein E1 (a Ub-activating enzyme) and the carboxyl terminals of Ub prior to the transfer to one of the existing E2 (Ub-conjugating enzyme) enzymes. There, E2s ligate with the Ub protein lig-

ase E3 enzymes before attaching onto target proteins. This process normally causes both mono- and multiple-Ub attachments to target proteins, and prepares them for degradation by proteasome [37].

Disrupted mitochondrial dynamics is intimately related to disturbed cellular homeostasis and is linked to accumulation of ROS and onset of various cardiac diseases, including cardiac hypertrophy, heart failure, alcoholic cardiomyopathy, dilated cardiomyopathy, and ischemic heart disease [37, 38]. For example, ROS accumulation in mitochondria is known to lead to increased ubiquitination of SOD2 in type 2 diabetic mice [39]. Ubiquitin ligases and de-ubiquitinating enzymes participate in the regulation of mitochondrial fission and fusion and directly contribute to the pathophysiology of heart diseases through the ubiquitin ligase seven in absentia homolog 2 (Siah2) [37]. Siah2 is a RING finger ligase to regulate prolyl hydroxylases PHD3 and PHD1 under hypoxia, thereby turning on the master regulator of hypoxia—hypoxia-inducible factor (HIF)-α. Recent studies have identified a role for the ubiquitin ligase Siah2 in the control of mitochondrial membrane protein degradation by proteasome, therefore protecting hearts against oxidative stress injury [36]. These authors found that hypoxia-induced mitochondrial fission is mediated by the mitochondrial scaffolding protein A kinase anchor protein 1 (AKAP121). AKAP121 may serve as a substrate of Siah2 for the maintenance of mitochondrial dynamics through modulation of the phosphorylation of dynamin-related protein 1 (Drp1) and the PKA-independent inhibition of the Drp1-Fis1 complex under cardiac stress. Ubiquitination and proteasomal degradation of these proteins were found to play a key role in the regulation of mitochondrial dynamics [37].

## 4. ACETYLATION

Lysine acetylation is a type of post-translational modifications to regulate mitochondrial homeostasis. Sirtuins, belonging to the family of class III histone deacetylases, require $NAD^+$ as a metabolic cofactor pertinent to enzymatic function, cellular stress response and, oxidative stress stimuli [40]. Sirtuins contain seven $NAD^+$-dependent protein deacetylases (Sirt1–7). Among them, mitochondrial Sirt3 plays a role in mitochondrial lysine acetylation and contributes to the antioxidant defense leading to preserved mitochondrial integrity. The beneficial effects of caloric restriction on oxidative stress are reported to rely on the antioxidant property of Sirt3 [41]. In response to stress, mitochondrial complex I deficiency leads to ROS accumulation and decreased $NAD^+$/NADH ratio in the heart, thus inhibiting Sirt3 activity and favoring protein acetylation [42]. Doxorubicin, a widely used anticancer drug, is known to induce cardiac mitochondrial damage through up-regulated ROS level. However, this anti-neoplastic agent triggered depletion of Sirt3, enormous acetylation of mitochondrial proteins, and mitochondrial DNA damage [43]. Along the same line, deletion of Sirt3 was associated with exacerbated cardiac hypertrophy in doxorubicin-induced dilated cardiomyopathy, and with increased cardiovascular risk factors in low-density lipoprotein (LDL) receptor-knockout mice [43, 44].

Manganese SOD (MnSOD, also known as SOD2) is the essential mitochondrial ROS scavenging enzyme that converts superoxide to hydrogen peroxide ($H_2O_2$). $H_2O_2$ is subsequently converted to water by catalase and other peroxidases. It was recently shown that MnSOD activity is tightly regulated by reversible acetylation of evolutionarily conserved lysine(s) [40]. Interestingly, overexpression of Sirt3 decreased cellular levels of acetylation and ROS, activated mitochondrial SOD, and up-regulated major ROS-detoxifying enzymes [1, 45]. Importantly, as a member of the sirtuin family of NAD-dependent deacetylases, Sirt3 is found to preserve cardiac function by way of mitochondrial SOD and transcription factor FoxO3A [41, 46].

## 5. OXIDATIVE MODIFICATION

Among all post-translational modifications, the oxidative post-translational modification (Ox-PTM) of mitochondrial proteins is perhaps the most likelihood event triggered by ROS [47]. Oxidation refers to a unique covalent PTM of products by ROS, or the secondary products from oxidative stress, which are produced by various metabolic processes [48]. Oxidation can occur in a number of amino acid residues, including histidine, asparagine, lysine, tyrosine, and cysteine. Ox-PTM of the cysteine residues is an essential event to alter protein structure and then cellular function. The exclusive feature of a cysteine side chain permits a range of Ox-PTMs, leading to multi-

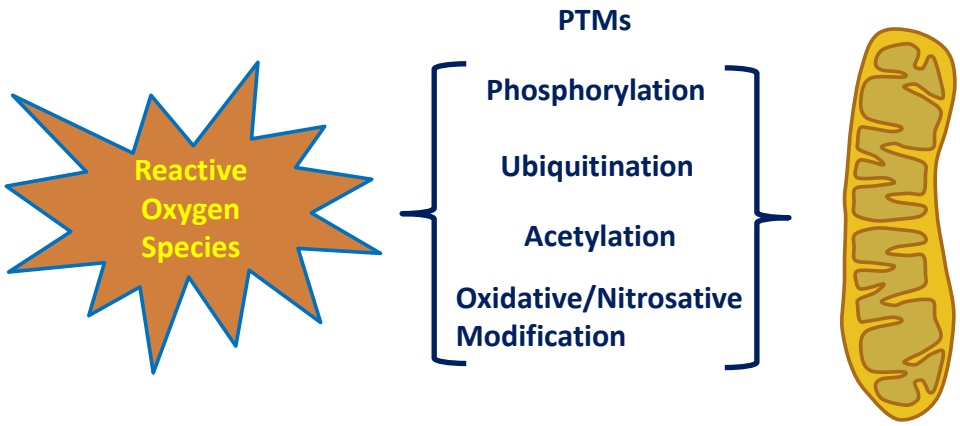


**FIGURE 1. Mitochondrial quality control via PTMs.** As illustrated, post translational modifications (PTMs) of proteins are critical for regulating mitochondrial biological activity, and they occur mostly via phosphorylation, ubiquitination, acetylation, and oxidative and nitrosative modification.

ple regulatory consequences. Up to date, the majority of cysteine Ox-PTMs involve the reversible reaction between diffusible small molecules and free thiol groups located on a cysteine side chain. These reactions may be reversed by the antioxidant enzyme (back to free thiols) or further converted to other Ox-PTMs dependent on the surrounding redox environment [49].

Oxidative protein modification plays a key role in the onset and development of heart failure [48, 49]. It is suggested that tryptophan residues in mitochondria can be the main targets of oxidative modification [50]. Not surprisingly, one hallmark for heart failure is progressive ROS production. Given that heart failure is associated with progressive ROS production, it is likely that mitochondrial quality control may serve as a safeguard for oxidative damage in cardiomyopathy and ultimately heart failure [49, 51].

## 6. NITROSATIVE MODIFICATION

Some post-translational modifications arise from the attachment of chemical components produced physiologically inside cells, such as nitric oxide (NO). NO acts as a second messenger, originating a wide range of reactive nitrogen species (RNS) [11]. NO-driven reactions can lead to several modification residues, such as the reaction of NO with ROS to form RNS switches from nitration to S-nitrosylation (SNO) of proteins. In fact, SNO of proteins is a specific PTM where the NO moiety covalently binds to the free cysteine residues of the target protein, which plays an critical part in heart health, especially within myocardial mitochondria [52]. It is understood that unstable levels of ROS/RNS are responsible for cardiac cytotoxicity, which then contribute to contractile dysfunction and heart failure. Indeed, SNO is of principal significance in either promoting or restricting ROS/RNS generation or subsequently reperfusion injury. Critical kinases may be turned on by ROS/RNS to elicit cardioprotection [53]. This process should be able to prevent mitochondrial transition pore opening and ROS-induced cytokine release. Finally, SNO may also represent a shield against oxidative stress, and thus nitrosylated protein may shield cells away from an irreversible oxidation and burst of ROS/RNS challenge [52].

In addition, the S-nitrosylated proteins work as a source of NO as intracellular signaling molecule in cardiomyocyte mitochondria [54]. It seems that most effects of NO are mediated by SNO of essential proteins, a covalent modification process between protein cysteine thiols and NO molecules to form S-nitrosothiol. Mitochondria were found to possess abundance of these tyrosine nitrated proteins as evidenced by enhanced 3-nitrotyrosine staining in the hearts following ischemia-reperfusion challenge [55]. This SNO process may be either an indication of PTM alteration or damaged mitochondrial quality

control in response to myocardial ischemia-reperfusion challenge [55, 56].

## 7. CONCLUSION

A steadily growing body of evidence points to an essential role of PTMs in mitochondrial homeostasis (**Figure 1**), due in part to the advance of new technologies. Nonetheless, accurate identification of the modified amino acid residues is still challenging at the present time. Such residue identifications are pertinent to the direct evaluation of the functional outcomes of any given PTMs, such as Ox-PTMs. In addition, elucidation of the precise enzymes responsible for the PTM mechanisms is also pivotal for the understanding of PTMs. This will lead to the possible advancement of therapies to manipulate PTMs along with mitochondrial function to combat against ROS over production and oxidative injury.

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