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Mechanistic Role of Kinases in the Regulation of Mitochondrial Fitness

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Abstract

Mounting evidence indicates that mitochondria contain multiple phosphorylation substrates and that protein kinases translocate into mitochondria, suggesting that protein phosphorylation in this organelle could be fundamental for the regulation of its own function. Here we examine the mechanistic role of cellular kinases in the fine regulation of key mitochondrial activities, including mitochondrial quality control, fission/fusion processes, metabolism, and mitophagy.

Mitochondrial Respiration

Mitochondria are cytoplasmic organelles that are involved in oxidative energy metabolism, producing the most of our cellular energy by oxidative phosphorylation [1, 2], through the metabolization of nutrients and production of ATP. Mitochondrial ATP production relies on the electron transport chain (ETC), composed of respiratory chain complexes I-IV.

The Regulation of Proteins in the ETC

SRC family kinases (SFKs) are the most involved in tyrosine phosphorylation of mitochondrial proteins to regulate mitochondrial respiration. Src kinases affect the enzymatic activities of complexes I, III-V; importantly, SRC phosphorylates different substrates in the ETC (Table 26.1). Indeed, Src is present inside mitochondria where it phosphorylates the subunit II of cytochrome c oxidase [3], increasing the enzymatic activity of complex IV. Moreover, adenine nucleotide translocase 1 (ANT1), which is able to transport ADP from cytosol within mitochondria in exchange of ATP, is known to be phosphorylated on Tyr¹⁹⁰ and Tyr¹⁹⁴ by c-Src kinase [4–6].

Src is also known to phosphorylate NDUFV2 (NADH dehydrogenase [ubiquinone] flavoprotein 2) of complex I at Tyr¹⁹³, and SDHA (succinate dehydrogenase A) of complex II at Tyr²¹⁵ [7]. NDUFV2 phosphorylation is required for NADH dehydrogenase activity, affecting respiration activity and cellular ATP content, while SDHA phosphorylation has no effect on enzyme activity, but affects reactive oxygen species production. Furthermore, the tyrosine protein kinase Fgr phosphorylates flavoprotein of succinate dehydrogenase at Tyr⁵³⁵ and Tyr⁵⁹⁶ and aconitase at Tyr⁷¹, Tyr⁵⁴⁴, and Tyr⁶⁶⁵ [8, 9].

Mitochondrial Biogenesis

Mitochondrial biogenesis can be defined as the growth and division of pre-existing mitochondria leading to a variation in number, size, and mass. It is dependent on different signaling cascades and transcriptional complexes that promote the formation and assembly of mitochondria.

The Regulation of PGC1 α

The peroxisome proliferator-activated receptor γ coactivator 1 (PGC1) family of transcriptional coactivators has recently emerged as central regulator of metabolism being a positive modulator of mitochondrial biogenesis and respiration [10]. PGC1 α is a co-transcriptional regulation factor that induces mitochondrial biogenesis by activating different transcription factors, including NRF-1 and NRF-2. These latter on turn activate Tfam to increase replication of mtDNA and to induce the transcription of key mitochondrial enzymes [11]. AMPK, p38 MAPK, and GSK3 β are the best-characterized protein kinases known to target PGC1 α . It has been shown that PGC1 α is increased in response to activation of AMPK and is reduced in both AMPK null [12] and dominant negative mice [13]. Indeed, AMPK directly phosphorylates PGC1 α on Thr177 and Ser538 [14] thereby enhancing its co-transcriptional activity and consequently mitochondrial gene expression. p38 MAPK phosphorylates PGC1 α at Thr²⁶², Ser²⁶⁵, and Thr²⁹⁸ in response to cytokine stimulation in muscle cells [15]. Moreover, it enhances the activity of PGC1 α by increasing its stability and by disrupting the inactivating interaction between PGC1 α and the co-repressor p160MBP in myoblasts [16]. Finally, PGC-1 α is also phosphorylated by glycogen synthase kinase 3 β (GSK3 β), which inhibits PGC1 α by enhancing its proteasomal degradation in the nucleus during oxidative stress [17].

Mitochondrial Quality Control

Mitochondria have an important role in the regulation of cell survival, cell death and metabolic homeostasis. They continuously fuse or divide to maintain their functions and damaged mitochondria after fission are removed through mitophagy. Thus, several mechanisms are involved in the regulation of mitochondrial quality control including mitochondrial fission and fusion [18], Parkin-dependent pathways [19] and degradation of damaged mitochondria by lysosomes [20] and autophagosomes [21].

The Regulation of Mitochondrial Fission

Mitochondria fission and fusion are mainly mediated by highly conserved guano-sine triphosphatases (GTPases) [22, 23]. Among them, dynamin-related protein 1 (DRP1) is the GTPase that regulates mitochondrial fission [24, 25]. It is a cytosolic protein that once activated translocates to the outer mitochondrial membrane where multimerizes in order to create a ring-like structure that constricts and divides the organelle [26, 27]. Drp1 activity is mainly regulated through phosphorylation in different sites by several protein kinases (Table 26.2). However, data on the effects of DRP1 phosphorylation are rather puzzling since different kinases phosphorylates the same site in DRP1 resulting in opposing effects, as described below.

PKA has a key role in the regulation of DRP1 activity by preventing its translocation to the mitochondria and inhibiting the process of mitochondrial fission. Indeed, Cribbs & Strack demonstrated that PKA phosphorylates Drp1 at Ser⁶⁵⁶ and this attenuates the GTPase activity of Drp1 promoting cell survival [28]. Chang & Blackstone discovered that PKA phosphorylates DRP1 also at Ser⁶³⁷ inhibiting its GTPase activity [29]. Accordingly, the phospho-mimetic substitution Ser637Asp blocks mitochondrial fission and apoptotic cell death [29]. Thus, PKA exerts an inhibitory effect on DRP1 activation by phosphorylation of both Ser⁶⁵⁶ and Ser⁶³⁷, even if no data are available to understand whether phosphorylation at these sites could have different physiological implications. On the contrary, phosphorylation of Ser⁶³⁷, which is inhibitory in PKA signaling, induced mitochondrial fission when is due to Ca²⁺/calmodulin-dependent protein kinase Ia (CaMKIa) [30]. Similarly, phosphorylation of DRP1 at Ser⁶¹⁶ by ERK2 activates DRP1 and promotes mitochondrial fission [31], whereas phosphorylation at the same residue by CDK5 exerts opposite effects [32]. Thus, the effects of DRP1 phosphorylation on mitochondrial fission depend on both the type of kinase and the specific phosphorylated residues. However, further studies are needed to clarify such effects.

Regulation of Mitochondrial Fusion

Mitofusins (MFNs) 1 and 2 are a class of conserved GTPases of the mitochondrial outer membrane that are essential for mitochondrial fusion and consequently to maintain normal mitochondrial morphology. MFNs are essential for normal cardiac function. Indeed, the combined deletion of MFN1 and MFN2 in murine hearts induces mitochondrial and cardiomyocyte dysfunction which rapidly leads to progressive and lethal dilated cardiomyopathy. MFN1 and 2 can be phosphorylated and such phosphorylation affects their ability to modulate mitochondrial fusion (Table 26.3).

Indeed, MFN2 was found to be phosphorylated by PINK1 at Thr¹¹¹ and S⁴⁴² to become a mitochondrial receptor for Parkin and eventually promote mitophagy. Moreover, MFN2 can be also phosphorylated by JNK at Ser27 causing its degradation through the ubiquitin-proteasome pathway which in turn affects both mitochondrial dynamics and apoptosis [33]. Also MFN1 can be phosphorylated at Thr⁵⁶² by ERK to modulate apoptotic responses [34]. Indeed, this inhibitory phosphorylation of MFN1 induces its association with BAK facilitating its oligomerization and inducing cytochrome *c* release and cell death [34].

Parkin-Dependent Mechanisms

PINK1 is a kinase associated with mitochondria: the loss of this kinase expression causes mitochondrial dysfunction and mitophagy [35–37]. Parkin is an E3 ubiquitin ligase suggested to be downstream of PINK1 to regulate the removal of damaged mitochondria. Indeed, PINK1 is activated by mitochondria membrane potential depolarization and is imported into mitochondria to activate Parkin [38–41]. This latter causes proteasomal degradation of outer mitochondrial membrane proteins [42, 43] and selective autophagy of damaged mitochondria [44], suggesting that PINK1 and Parkin mediate a mitochondrial quality control pathway. The removal of damaged mitochondria was thought to be mainly attributable to the activation of PINK1-Parkin-Ubiquitin cascade: PINK1 directly phosphorylates Parkin at Ser⁶⁵ which on turn activates Ubiquitin [45]. Actually, recent findings changed this view: PINK1 has been shown to recruit Parkin to mitochondria also in presence of mutation of Ser⁶⁵ to Alanine suggesting the ability of PINK1 to regulate Parkin in a phosphorylation independent manner. Moreover, PINK1 directly phosphorylates Ubiquitin at Ser⁶⁵ which on turn activates Parkin [46]. Thus, PINK1 phosphorylates at Ser⁶⁵ both Parkin and Ubiquitin to induce the full activation of Parkin, as summarized in Fig. 26.1.

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Fig. 26.1.

The full activation of Parkin is dependent on PINK1-mediated phosphorylation of both Parkin and ubiquitin at Ser⁶⁵

Table 26.1

Substrates of SRC in the ETC e the effects of substrate phosphorylation

SRC substrates	Phosphorylation sites	Effect
ANT1	Tyr ¹⁹⁰ and Tyr ¹⁹⁴	Protection of cardiac mitochondria against ischemic-reperfusion injury
Cytochrome c oxidase	unknown	Increase of the enzymatic activity of complex IV
NDUFV2	Tyr ¹⁹³	Increase of NADH dehydrogenase activity
SDHA	Tyr ²¹⁵	Regulation of reactive oxygen species production

Table 26.2

Protein kinases that phosphorylate DRP1 and their effects on DRP1 activation

Protein kinases	DRP1 phosphorylation sites	Effect on DRP1
PKA	Ser ⁶⁵⁶	Inhibition
	Ser ⁶³⁷	Inhibition
CaMK1 α	Ser ⁶³⁷	Activation
ERK	Ser ⁶¹⁶	Activation
CDK	Ser ⁶¹⁶	Inhibition

Table 26.3

Mitofusins phosphorylation sites and effects of phosphorylation on mitophagy

Protein kinases	MFN phosphorylation sites	Effect on mitophagy
PINK1	T ¹¹¹ of MFN2	Activation
PINK1	S ⁴⁴² of MFN2	Activation
JNK	Ser ²⁷ of MFN2	Inhibition
ERK	T ⁵⁶² of MFN1	Inhibition