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Mitochondrial TFAM as a Signaling Regulator between Cellular Organelles: A Perspective on Metabolic Diseases

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Tissues actively involved in energy metabolism are more likely to face metabolic challenges from bioenergetic substrates and are susceptible to mitochondrial dysfunction, leading to metabolic diseases. The mitochondria receive signals regarding the metabolic states in cells and transmit them to the nucleus or endoplasmic reticulum (ER) using calcium (Ca^{2+}) for appropriate responses. Overflux of Ca^{2+} in the mitochondria or dysregulation of the signaling to the nucleus and ER could increase the incidence of metabolic diseases including insulin resistance and type 2 diabetes mellitus. Mitochondrial transcription factor A (Tfam) may regulate Ca^{2+} flux via changing the mitochondrial membrane potential and signals to other organelles such as the nucleus and ER. Since Tfam is involved in metabolic function in the mitochondria, here, we discuss the contribution of Tfam in coordinating mitochondria-ER activities for Ca^{2+} flux and describe the mechanisms by which Tfam affects mitochondrial Ca^{2+} flux in response to metabolic challenges.

Keywords: Calcium; Cell nucleus; Diabetes mellitus, type 2; Endoplasmic reticulum; Mitochondria; TFAM protein

INTRODUCTION

Tissues that are very active in energy metabolism are more likely to face metabolic challenges from the bioenergetic substrate and are susceptible to mitochondrial diseases [1]. Several genes from both the nucleus and mitochondria are required for mitochondrial formation and maturity. Thus, intercommunication between the mitochondria and nucleus is critical for highly metabolic tissues such as skeletal muscle, heart, liver, and adipose tissue.

The mitochondria are heavily involved in diabetes, insulin resistance, complications derived from diabetes [2], and cellular calcium (Ca^{2+}) flux, an important process for mitochondrial function and metabolic diseases since Ca^{2+} signaling regulates various events related to cell death and energy metabolism [3-7]. Mitochondrial metabolic dysfunctions induced by insulin resistance are linked to abnormal Ca^{2+} flux and high reactive oxygen species (ROS) levels. The metabolic functions that regulate Ca^{2+} flux are also associated with glucose uptake and metabolism [4]. The nucleus responds to Ca^{2+} signals, yet the endoplasmic reticulum (ER) dynamically interacts with the mitochondria and regulates Ca^{2+} signaling. This process is essential for several metabolic processes and physiological functions [8], and is important for regulating normal mitochondrial biogenesis [5,9] and glucose metabolism [4].

The nucleus encodes the mitochondrial transcription factor

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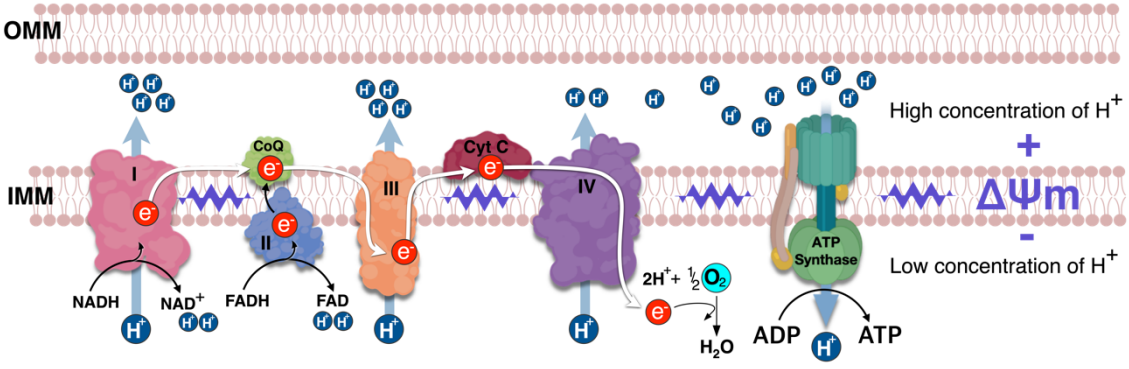
A (TFAM), which activates transcription in the mitochondria and transmits a signal to the nucleus based on the mitochondrial state by regulating Ca^{2+} levels. It has been shown that it also prevents high-fat diet-induced insulin resistance [10]. *Tfam*-mutant mice developed diabetes and exhibited mitochondrial deoxyribonucleic acid (mtDNA) depletion, deficient oxidative phosphorylation, and abnormal mitochondrial structure [9]. This suggests that TFAM is involved in cellular metabolic function, possibly involving Ca^{2+} signaling.

This review focuses on how mitochondrial TFAM regulates Ca^{2+} signaling and how dysregulation of Ca^{2+} by the mitochondria leads to metabolic disease in type 2 diabetes mellitus (T2DM).

MITOCHONDRIA AND TFAM

Mitochondria play a key role in cellular physiology, and are responsible for producing cellular energy and essential metabolites, along with regulating apoptosis [11]. These functions are dependent on gene expression in both the mitochondria and nucleus and are regulated by communication between mitochondria and other organelles.

Biosynthesis of each electron transport chain (ETC) complex in the mitochondria is jointly regulated by the nucleus and mtDNA. Since several genes are required for mitochondrial biogenesis (Fig. 1), TFAM is an important regulator between the mitochondria and nucleus, since Tfam is expressed from the nucleus but acts on the mitochondria. TFAM regulates transcription of the 13 genes for ETC protein, 22 for transfer RNAs, and two for ribosomal RNAs encoded by mtDNA [12,13]. TFAM binds to mtDNA and enhances transcription in association with mitochondrial RNA polymerase and either mitochondrial transcription factor B1 (TFB1M) or B2 (TFB2M) [14]. In addition to binding to the specific region of the promoter, TFAM binds nonspecifically to random sites on mtDNA [15,16] which enhances the stabilization and maintenance of the mitochondrial chromosome [15-17] as well as regulation of mtDNA copy number [18]. Interestingly, since TFAM overexpression has been shown to increase mtDNA without alteration of respiratory capacity and mitochondrial biogenesis



complex I	complex II	complex III	complex IV	ATP synthase	
7	1	0	3	2	mtDNA
39	10	4	10	14	nDNA

Fig. 1. Schematic illustration of the mechanism underlying the mitochondrial membrane potential (ΔΨm) and electron transport chain (ETC) as well as a summary of subunits of the four-ETCs and adenosine triphosphate (ATP) synthase (complex V) encoded by nuclear and mitochondrial genes. The five complexes are embedded in the inner mitochondrial membrane. ΔΨm is generated via a proton pump comprising complex I, III, and IV, and the electron is sequentially transferred from complex I to IV, referred to as the ETC. The proton pump-induced proton gradient between the intermembrane space and matrix generates ΔΨm. The flow of protons through ATP synthase from the intermembrane space to the matrix is coupled with ATP synthesis. Indicated below each complex is the number of protein subunits encoded by nuclear (nDNA) and mitochondrial genomes (mtDNA). OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; NAD(H), nicotinamide adenine dinucleotide; CoQ, coenzyme Q; FAD(H), flavin adenine dinucleotide; Cyt C, cytochrome C; ADP, adenosine diphosphate .

[10], it seems that the mechanisms underlying mitochondrial biogenesis and maintenance of mtDNA by TFAM are separately regulated. Moreover, TFAM overexpression increases the nuclear expression of various factors associated with Ca^{2+} signaling and glucose metabolism in mouse skeletal muscle [10]. TFAM is also involved in mitochondrial function including lower ROS via enhancing antioxidants, 5' adenosine monophosphate-activated protein kinase (AMPK) activation, and mitochondrial uncoupling as well as regulation of membrane potential [10]. Therefore, intercommunication between the mitochondria and nucleus is closely regulated to cope with a sudden change in cellular energy challenges, with TFAM possibly playing an important role in this interaction.

NUCLEUS DISPATCHES TFAM TO THE MITOCHONDRIA

Signaling from the nucleus can regulate mitochondrial gene expression or mtDNA replication in response to cellular metabolic challenges or environmental signals [19], via TFAM. Nuclear respiratory factor 1 (NRF-1) can bind specific promoters of various nuclear genes required for mitochondrial respiratory function [20], which coordinates the expression of respiratory subunits with the mitochondrial transcriptional system [13]. NRF-1 also binds and activates the *Tfam* promoters [21], thereby inducing a cellular signaling cascade in skeletal muscle in response to exercise training [22,23]. In this context, Ca^{2+} treatment in muscle cell increases TFAM and NRF-1 protein levels as well as mitochondrial biogenesis [5,9], suggesting Ca^{2+} as a signaling molecule between cellular organelles. Redox reactions are activated in the mitochondria during exercise-induced oxidative phosphorylation and the incidence of NRF-1 binding to the TFAM promoter is increased under pro-oxidant conditions. However, *Tfam* expression is inhibited by deactivated NRF-1 [24], suggesting that Tfam expression mediated by NRF-1 is regulated under activated redox conditions. Interestingly, mtDNA depletion induces an increase in both along with oxidative stress [25]. NRF-1 and *Tfam* mRNAs are also increased in response to mitochondrial lipopolysaccharide-induced oxidative damage [26], suggesting that TFAM is increased to mitigate these cellular conditions, which, along with NRF-1, have been shown to decrease ROS via enhanced antioxidant and mitochondrial uncoupling [10].

Taken together, when the cellular environment undergoes metabolic challenge, including exercise or metabolic alteration, NRF-1 and TFAM are upregulated in the nucleus followed by TFAM translocation into the mitochondria to mitigate this change by regulating mitochondrial oxidative phosphorylation and redox valence.

Intercommunication between mitochondria and ER for intracellular Ca^{2+} flux

Ca^{2+} signaling and flux regulate numerous cellular physiological processes, including neuronal excitability, muscle contraction, nuclear gene expression, and mitochondrial integrity, function, and dynamics [27]. Accumulated data have shown that mitochondrial Ca^{2+} content is low under basal conditions, while increases in cytosolic free Ca^{2+} in response to various agents (nutrients, hormones, neurotransmitters, etc.) elevates mitochondrial Ca^{2+} levels [28,29]. This enhances the activity of tricarboxylic acid (TCA) cycle dehydrogenase (pyruvate-, isocitrate-, and α-ketoglutarate dehydrogenase) required for oxidative phosphorylation [30]. Thus, activation of oxidative metabolism via enhanced Ca^{2+} flux can increase the supply of redox cofactors, such as a reduced form of nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) to drive ETC and adenosine triphosphate (ATP) synthesis (Fig. 2).

TFAM regulates the mitochondrial membrane potential ($\Delta\Psi m$) and increases calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ) [10]. In turn, $\Delta\Psi m$ can regulate cellular Ca^{2+} flux [31], while CaMKKβ is activated in a calcium-dependent manner [32]. Thus, TFAM-driven $\Delta\Psi m$ controls Ca^{2+} flux in the cell via various Ca^{2+} channels. Perturbation of TFAM expression cannot protect mtDNA [18], while damaged mtDNA has been reported to increase ER stress [33]. This suggests that TFAM may be involved in the mitochondria-ER interactions.

The lumen of the ER is the main storage site of Ca^{2+} and is constantly refilled via the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump [34,35]. When skeletal muscle contraction is initiated, cytosolic Ca^{2+} concentration is rapidly increased by 3 to 4-fold, with the sarcoplasmic reticulum (SR), an ER in skeletal muscle, optimizing the coupling of excitation and contraction of muscle fibers. SR has channels for efflux of Ca^{2+} into the cytosol in response to depolarization, while SERCA transports cytosolic Ca^{2+} in an ATP-dependent manner into the ER lumen, thereby terminating the contraction. The close link between the mitochondria and SR/ER allows for rapid and potent local Ca^{2+} signaling (Fig. 2) [6,36].

Mitochondrial and endoplasmic reticulum contacts (MERC)

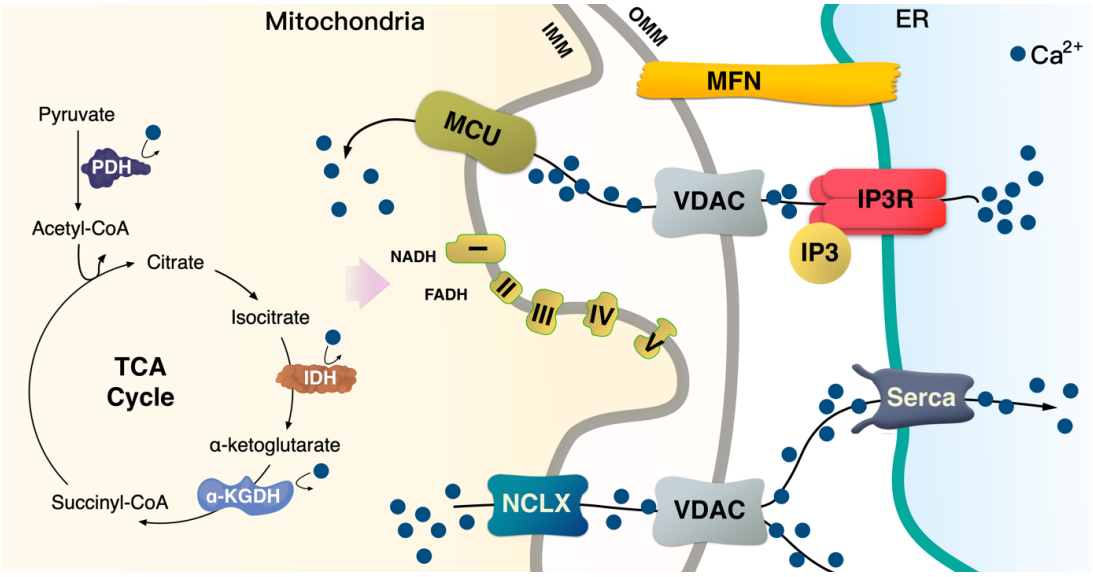


Fig. 2. Mitochondria contacts endoplasmic reticulum (ER) and regulates cellular calcium (Ca^{2+}) flux. Na^{+}/Ca^{2+} exchanger (NCLX) regulates Ca^{2+} efflux from the mitochondria to the cytosol. The sarco/endoplasmic reticulum Ca^{2+} ATPase (Serca) in the ER membrane can take up Ca^{2+} into the ER lumen. Inositol triphosphate (IP3) binds to its own receptor (IP3R) leading to ER Ca^{2+} efflux in the cytosol or the mitochondria via a voltage-dependent anion channel (VDAC) at the outer mitochondrial membrane (OMM); in turn, the mitochondrial calcium uniporter (MCU) transfers it across the inner mitochondrial membrane (IMM). Ca^{2+} is necessary for the activation of pyruvate dehydrogenase (PDH), isocitrate dehydrogenase (IDH), and α-ketoglutarate (KGDH); thus, Ca^{2+} flux in the mitochondria is a key process for the tricarboxylic acid (TCA) cycle to provide nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH) for the electron transport chain (ETC). Mitofusin (MFN) induces mitochondria-ER physical tethering to provide a stable association.

orchestrate cellular physiological functions such as mitochondrial Ca^{2+} signaling and dynamics. Under physiological stimulation requiring intercommunication between mitochondria and ER, mitofusin 2 (MFN2) tethers to stabilize both organelles [31,37,38]. Meanwhile, inositol 1,4,5-trisphosphate (IP3) binds to the IP3 receptor (IP3R), triggering Ca^{2+} release from the ER through IP3R, which can directly release Ca^{2+} into the cytosol or mitochondria [36]. Released Ca^{2+} from the ER enters the mitochondrial intermembrane space by first passing through the outer mitochondrial membrane via the voltage-dependent anion channel (VDAC) [39], then the mitochondrial calcium uniporter (MCU) transporter across the inner mitochondrial membrane [40,41]. TFAM is linked to Ca^{2+} regulation via MFN2 [42]. Ca^{2+} flux is essential for mitochondrial bioenergetic processes, which is linked to the ΔΨm (Figs. 1 and 2).

TFAM regulates the mitochondrial and ER Ca^{2+} signaling to nucleus via ΔΨm

Mitochondria are key organelles in Ca^{2+} flux regulation in cells. While Ca^{2+} flux in mitochondria is one of the most significant processes in regulating energy production and communication with other organelles, it mediates various pathologies associated with metabolic disease.

Mitochondrial Ca^{2+} uptake is directly linked to mitochondrial bioenergetics, where depletion of mitochondrial ΔΨm abrogates mitochondrial Ca^{2+} uptake, and defects in the respiratory chain have been linked to the decreased ability of mitochondria to pump Ca^{2+}. Energetic substrates derived by the TCA cycle are serially reduced to equivalents by ETC, and these redox reactions are coupled with protons pumping from the matrix into the intermembrane space [43]. The proton electrochemical gradient induced by ΔΨm and pH gradient is necessary to produce ATP (Fig. 1). Thus, ΔΨm can maintain mitochondrial Ca^{2+} uptake and physiological functions (Figs. 1 and 2) [44,45].

Lack of TFAM decreases Serca2a expression from the nucleus [46], indicating that TFAM in the mitochondria signals to the nucleus to transcribe *Serca2a* and coordinate with ER to maintain Ca^{2+} homeostasis. The ER can regulate the Ca^{2+} efflux channels [36], which could affect cytosolic Ca^{2+} and force dur-

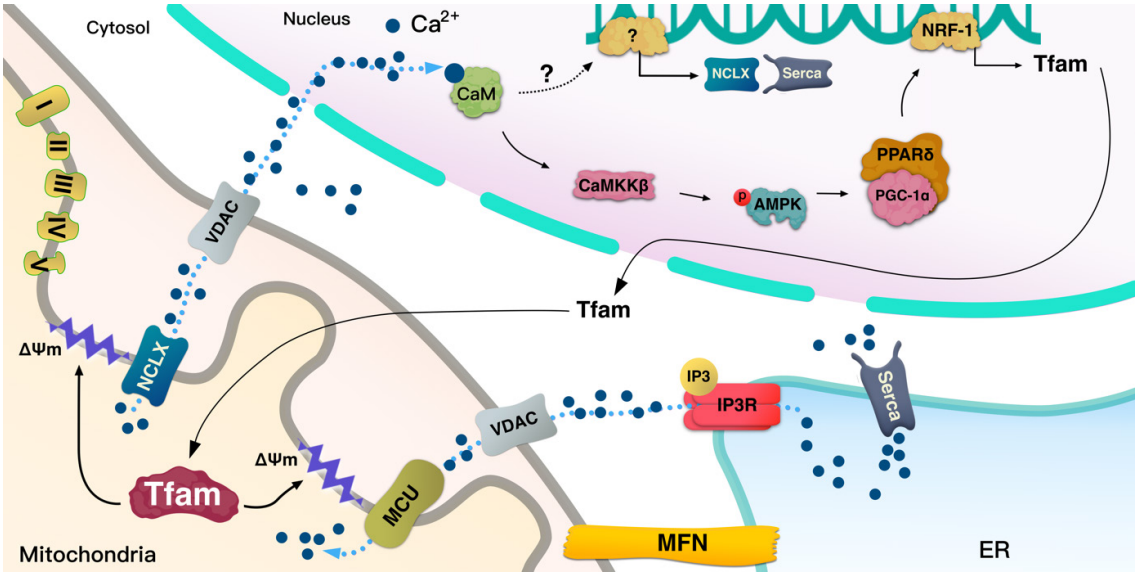


Fig. 3. A schematic illustration of the hypothesized role of mitochondrial transcription factor A (Tfam). Mechanism by which Tfam sends calcium (Ca^{2+}) signals to the nucleus. Na^+/Ca^{2+} exchanger (NCLX) and mitochondrial calcium uniporter (MCU) are embedded at the inner mitochondrial membrane (IMM). The activity of these channels can be affected by ΔΨm flux, which can be regulated by Tfam. Thus, Tfam-driven ΔΨm can transmit Ca^{2+} signals via NCLX/voltage-dependent anion channel (VDAC) to the nucleus. Tfam can regulate the transcription of NCLX and sarco/endoplasmic reticulum Ca^{2+} ATPase (Serca); however, the specific mechanism is not clear in metabolic cells. Tfam increases calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ), 5' adenosine monophosphate-activated protein kinase (AMPK) phosphorylation, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), nuclear respiratory factor 1 (NRF-1), and peroxisome proliferator-activated receptor delta (PPARδ). The Tfam-induced increase in these proteins seems to be regulated by Ca^{2+} signaling. ER, endoplasmic reticulum.

ing contraction; hence, a reduced ER Ca^{2+} level in TFAM knockout muscle results in a decrease in cytosolic Ca^{2+} [47], which leads to a lower force in single fibers during contraction [47]. In TFAM knockout mice, an increase in mitochondrial Ca^{2+} occurs during repeated contraction [47], which may induce Ca^{2+} overload in the mitochondria and oxidative stress. In contrast with TFAM knockouts, *Serca2* gene can enhance contractile function and restore electrical stability in a heart failure model caused by ER(SR) Ca^{2+} leak [48]. Watanabe et al. [46] have shown that TFAM regulates *Serca2* gene transcription; however, it is not clear how TFAM in mitochondria regulates nuclear *Serca2a* transcription (Fig. 3).

As mentioned above, mice with muscle-specific deletion of TFAM exhibit Ca^{2+} overload in the mitochondria, which might be caused by the low Ca^{2+} efflux into the cytosol by the mitochondria. MCU is the main channel transporting Ca^{2+} from the cytosol, and Na^+/Ca^{2+} exchanger (NCLX) regulates efflux of Ca^{2+} from mitochondria, both of which are located at the inner mitochondrial membrane where they coordinate to regulate mitochondrial Ca^{2+} homeostasis. Mice with cardiac-specific deletion of TFAM showed how a failure in the coordination between MCU and NCLX in mitochondria can lead to cardiomyopathy [49]. TFAM deletion in cardiac muscle decrease Ca^{2+} efflux from mitochondria due to low NCLX gene and protein levels, with the reduced efflux potentially inducing mitochondrial Ca^{2+} overload [49]. Ca^{2+} flux through the MCU can be regulated by the voltage gradient across the inner mitochondrial membrane, while the opening probability of MCU is decreased based on ΔΨm depolarization [50]. Moreover, protonophore-induced depolarization of ΔΨm almost fully suppresses Ca^{2+} influx into the mitochondria [51]. These findings indicate that the large electrical driving force that arises from the negative potential across the inner ΔΨm is a major factor in regulating the influx of Ca^{2+} through MCU (Fig. 3).

Depolarization of ΔΨm or lack of mtDNA prevents mitochondrial Ca^{2+} uptake, elevating the cytoplasmic Ca^{2+} level and leading to mitochondrial retrograde signaling into the nucleus to activate Ca^{2+}-mediated transcription mechanisms involved in calcineurin and Ca^{2+}/calmodulin-dependent protein kinase (CaMK) [44,45]. Human TFAM (hTFAM) transgenic mice ex-

hibit mild ΔΨm uncoupling when fatty acids are used as a substrate for skeletal muscle mitochondria, despite mtDNA in skeletal muscle being increased by hTfam [10]. These alterations by hTfam result in an increase in mild uncoupling in ΔΨm, leading to an increase in CaMKKβ expression from the nucleus [10]. Therefore, TFAM regulates ΔΨm and mediates retrograde signaling to the nucleus by Ca^{2+} transmission. Interestingly, mild uncoupling of ΔΨm by hTFAM decreased ROS [10]. Since a lack of TFAM can mediate Ca^{2+} overload in the cytoplasm, it not only induces retrograde signaling to the nucleus but also increases ROS and apoptosis [45]. ROS produced by lack of TFAM in cells may be induced by an overload of Ca^{2+} [45]; however, hTFAM overexpression can decrease ROS and oxidative stress in tissue with mild uncoupling of ΔΨm [10]. Therefore, TFAM may mediate mild uncoupling of ΔΨm that regulates Ca^{2+} retrograde signaling to tightly control cellular ROS (Fig. 3).

The nuclear genome encodes approximately 1,500 proteins that are necessary for mitochondrial function and integrity [52,53]. Intercommunication between mtDNA and the nuclear genome is necessary for mitochondrial biogenesis and normal function. To facilitate the interplay between the organelles, TFAM functions signaling transmitter to the mitochondria from the nucleus, and sends signals back to the nucleus according to the state of the mitochondria by regulating Ca^{2+}. This signaling pathway is important for the regulation of normal mitochondrial biogenesis via peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), which orchestrates various transcription factors including NRF-1 and peroxisome proliferator-activated receptor delta (PPARδ) (Fig. 3).

Intracellular Ca^{2+} binds CaM, thereby mediating various cellular functions [3,54]. In skeletal muscle, this Ca^{2+}/CaM complex contract the muscle fiber [55]. The activation of Ca^{2+}/CaMKKβ enhances AMPK activation [32], which facilitates mitochondrial biogenesis via enhanced expression of PGC-1α [56]. Muscle-specific hTFAM transgenic mice exhibit higher levels of CaMKKβ and activated AMPK [10]. To maintain and manage mtDNA as well as to regulate mitochondrial function, it is also necessary to increase the amount of TFAM as the mitochondrial number increases. It has been shown that TFAM overexpression in skeletal muscle enhances the expression of NRF-1, which is an upstream transcription factor for itself [10] and PPARδ expression. PPARδ regulates glucose transporter type 4 (GLUT4) expression and glucose uptake in muscle tissue [57]. A higher level of PGC-1α and PPARδ in tissue is beneficial in improving metabolic diseases such as insulin resistance and T2DM (Figs. 3 and 4).

Ca^{2+} flux in metabolic disease

Skeletal muscle, liver, and adipose are metabolic and insulin-sensitive tissues. Mitochondrial dysfunction in those tissues is associated with lower levels of TFAM in various metabolic diseases such as obesity, insulin resistance, and T2DM, which mediate abnormal Ca^{2+} flux.

Role of MERC in diabetes

Tethering between mitochondria and ER plays a key role in Ca^{2+} homeostasis, which regulates energy metabolism, transportation of lipids, and apoptosis [58]. The influx of Ca^{2+} mediates insulin-stimulated glucose uptake in skeletal muscle [4]. The authors reported that a decrease in Ca^{2+} influx by IP3R inhibition improved insulin-stimulated glucose uptake in skeletal muscle without AKT signaling [4]. In adipocytes, cytosolic Ca^{2+} levels were increased by insulin stimulation [59]. The inhibition of Ca^{2+} signaling in adipocytes [59] and lack of IP3R in primary rat cardiomyocytes [60] decreased GLUT4 translocation and glucose uptake upon insulin stimulation, respectively. Moreover, mitochondrial dysfunction [61], dysregulation of lipid and Ca^{2+} homeostasis [62], and ER stress [63] have been reported to be closely linked to insulin resistance in the liver. Palmitate is known to reduce insulin sensitivity; it has been shown that disruption of the interaction between mitochondria and ER by palmitate induces insulin resistance in human's and mice's hepatocytes. However, an increase in these associations between organelles can prevent palmitate-induced insulin resistance [64]. Loss of IP3R1 decreases MERC formation and induces mitochondrial dysfunction and insulin resistance; however, restoration of MERC has been shown to improve palmitate-induced insulin resistance in hepatocytes [64]. It has been reported that AKT phosphorylates the IP3R channel, resulting in decreased Ca^{2+} release capacity through IP3R [65,66]. Moreover, loss of IP3R activity has been shown to change the integrity of MERC [64]. Although defects in MERC have been suggested to play a role in insulin resistance and T2DM [37,38,42], MERC-mediated Ca^{2+} flux for glucose homeostasis is complicated and tightly regulated; hence, other specific mechanisms might be involved in this process. Thus, further studies are required to demonstrate the relationship between insulin resistance and MERC, as well as the mechanisms involved in this process. ER-associated mitochondrial

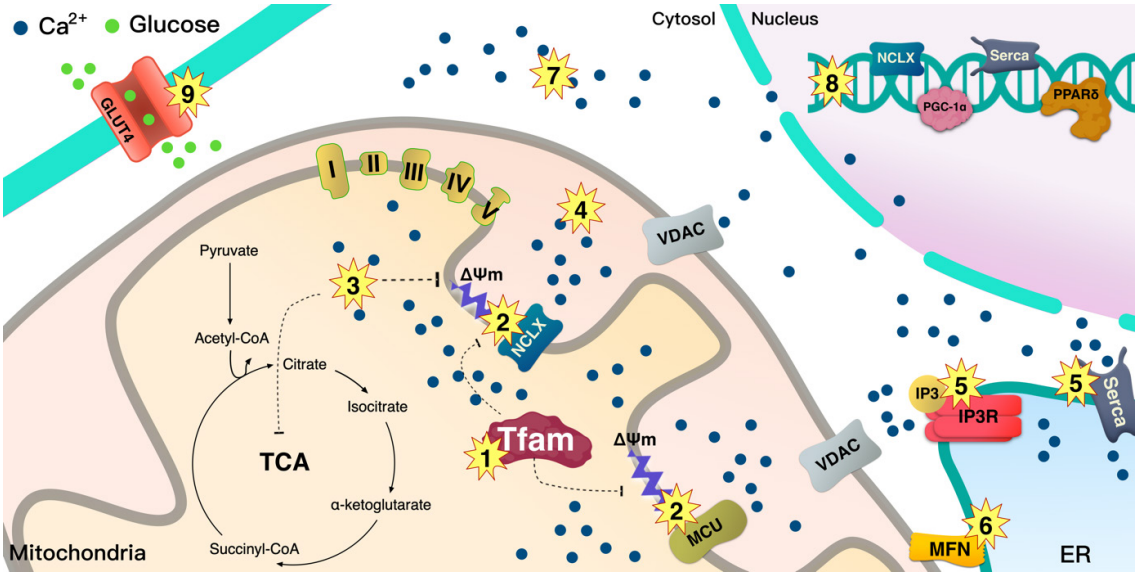


Fig. 4. A schematic illustration of the summary of the dysregulation of mitochondrial calcium (Ca^{2+}) flux in type 2 diabetes mellitus (T2DM). Lower mitochondrial transcription factor A (Tfam) levels in T2DM may serially induce mitochondrial Ca^{2+} dysregulation through $\Delta\Psi m/Ca^{2+}$ channels. (1) Lower Tfam levels in mitochondria clearly reduce the mitochondrial complex that regulates proton pumps, (2) leading to dysregulation of $\Delta\Psi m$ that controls the Na^{+}/Ca^{2+} exchanger (NCLX) channel. (3) Overload of Ca^{2+} induced by NCLX decreases tricarboxylic acid (TCA) cycle activity, resulting in reduced supply of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH) for the electron transport chain (ETC) proton pump; especially, overloaded Ca^{2+} increases inner mitochondrial membrane (IMM) depolarization and decreases $\Delta\Psi m$ for NCLX activity. Overloaded Ca^{2+} in (3) matrix and (4) intermembrane space may trigger apoptosis. (5) Dysregulation of Ca^{2+} by inositol triphosphate receptor (IP3R) and sarco/endoplasmic reticulum Ca^{2+} ATPase (Serca) is clearly linked to mitochondrial dysfunction and metabolic disease. (6) Loss of mitofusin (MFN) downregulates stable contact of the mitochondria and endoplasmic reticulum (ER), leading to T2DM. (7) Overloaded Ca^{2+} in cytosol also increases the incidence of T2DM. (8) Loss of Ca^{2+} signaling into the nucleus from the mitochondria may inhibit the transcription of NCLX, Serca, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), and peroxisome proliferator-activated receptor delta (PPARδ), (9) glucose transporter type 4 (GLUT4) expression is regulated by PPARδ. This can be a caused by a metabolic disease. MCU, mitochondrial calcium uniporter; VDAC, voltage-dependent anion channel.

division sites are spatially linked to mitochondrial nucleoids, which suggests a specific role for mitochondria-ER contacts in mtDNA maintenance [67]. ER-mitochondria contacts coordinate mtDNA synthesis with division to distribute newly replicated nucleoids into daughter mitochondria [68]. MFNs, proteins acting between mitochondria and ER [69], are also involved in metabolic diseases. Mitochondrial dysfunction is reportedly caused by depletion and point mutations of mtDNA in mice with muscle-specific deletion of MFN1 and MFN2 [69], while MFN2 expression in skeletal muscle is lower in patients with obesity or T2DM [37]. In contrast, overexpression of MFN2 has been shown to improve diet-induced insulin resistance [70], and MFN2 is necessary for normal glucose homeostasis [38]. Whether TFAM is directly involved in the process of forming or maintaining ER-mitochondria contacts remains to be determined, however, the loss of MFN1 and MFN2 decreases mtDNA that is maintained by TFAM [69], while PGC-1α, a transcription factor for *Tfam*, can increase the MFN2 expression levels via estrogen-related receptor-α (ERRα) [71]. Moreover, TFAM is involved in packaging mtDNA into a nucleoid [72-74]; hence, it is possible that TFAM indirectly participates in this process of MERC-mediated glucose homeostasis. hTFAM overexpression in skeletal muscle has been reported to prevent high-fat diet-induced insulin resistance along with preserving higher levels of mtDNA [10].

SERCA tightly regulates cytosolic Ca^{2+}, leading to glucose oxidation [75]. An increase in SERCA has been reported to improve diabetic cardiomyopathy [7,76]. A high-fat diet decreases *Serca2a* expression due to a lack of TFAM; however, overexpression of TFAM inhibits hydrogen peroxide-induced

decrease of *Serca2a* mRNA [46]. An increase in SERCA2a by TFAM can reduce levels of cytoplasmic Ca^{2+} and calpain and mitochondrial apoptosis factor, thereby improving glucose uptake in cardiac muscle [77]. Increased expression of Serca improves metabolic syndrome [78].

Role of mitochondrial Ca^{2+} regulation in T2DM

The MCU complex consists of a pore-forming MCU subunit, a regulatory subunit, and mitochondrial Ca^{2+} uptake proteins 1-3 (MICU1-3), which regulate the channel activity [79-82]. Indeed, a splicing variant of muscle-specific MICU1 with higher Ca^{2+} affinity facilitates mitochondrial Ca^{2+} uptake and ATP production, which is required for muscle contraction [83], whereas a deletion of MCU in skeletal muscle in mice decreases muscle force, indicating that through regulating Ca^{2+} uptake, MCU plays a vital role in energy production for contraction [84]. Deletion of the muscle-specific MCU induced lower activity of pyruvate dehydrogenase (PDH), which is sufficient to shift the preference of the substrate toward fatty acids from carbohydrates [85] since PDH converts pyruvate to acetyl-CoA. In addition, loss of MCU causes defects in Ca^{2+}-sensitive TCA cycle enzymes such as isocitrate and α-ketoglutarate dehydrogenases. Therefore, MCU-deleted mitochondria in skeletal muscle cannot sustain respiration without TCA cycle support, although MCU-deleted muscle mostly relies on fatty acids as substrate for mitochondrial respiration [85]. Thus, fatty acids do not completely oxidize and accumulate in muscle, leading to insulin resistance in skeletal muscle [86]. MCU is on the mitochondrial inner membrane that is dependent on ΔΨm, and hTFAM overexpression in skeletal muscle induces mild uncoupling of ΔΨm, which may change mitochondrial Ca^{2+} flux. This alteration shifts the preferred substrate for mitochondrial respiration from glucose to fatty acids [10]. However, the hTFAM transgenic model is different from the MCU deletion since muscle-specific hTFAM overexpression increases glucose uptake and prevents high-fat diet-induced insulin resistance (Figs. 3 and 4) [10].

The NCLX plays a vital role in mitochondrial efflux into the cytosol [87]. A decrease in NCLX function leads to the accumulation of Ca^{2+} in mitochondria, while impaired NCLX function has been reported in diabetic rat hearts [87,88]. A previous study has shown that deletion of TFAM in mouse cardiomyocytes decreases NCLX transcription [49], and TFAM regulates ΔΨm in skeletal muscle; therefore, TFAM seems to clearly link to mitochondrial Ca^{2+} efflux via ΔΨm (Figs. 3 and 4).

Overloaded Ca^{2+} levels have been reported in adipocytes from patients with obesity with insulin resistance [89] and from T2DM rats [90], which may link to the abnormal functions of mitochondria and ER which are unable to maintain Ca^{2+} homeostasis in cells. ΔΨm depolarization enhances ROS production [91-93]; however, hTFAM overexpression prevents fatty acid-induced ΔΨm depolarization in muscle cells and blocks ROS production in mouse skeletal muscle [10]. Moreover, mild ΔΨm uncoupling induced by hTFAM increases glucose uptake in skeletal muscle and improves high-fat diet-induced insulin resistance [10]. Further studies are required to determine how hTFAM regulates Ca^{2+} flux in mitochondria via ΔΨm.

CONCLUSIONS

This review has provided an overview of how mitochondria signaling to the nucleus and interact with the ER to regulate Ca^{2+} as well as the role of mitochondrial dysregulation via cellular Ca^{2+} homeostasis in the pathogenesis of metabolic diseases, including insulin resistance and T2DM. Although further investigation is required to determine mechanisms by which TFAM controls ΔΨm to regulate Ca^{2+} flux in the cell, accumulated data indicate that TFAM-driven ΔΨm regulates mitochondrial and ER's Ca^{2+} flux. This leads to Ca^{2+} signaling into the nucleus, thereby inducing the expression of various genes such as flux channels and signaling co-factors for Ca^{2+}. TFAM-mediated regulation of ΔΨm prevents high-fat diet-induced oxidative stress and insulin resistance via enhanced expression of GLUT4, PGC-1α, and PPARδ from the nucleus. Moreover, the mitochondria interact with the ER and regulate cellular Ca^{2+} flux. This process clearly influences mitochondrial TCA cycle and oxidative phosphorylation, whereas dysregulation of this process could increase metabolic diseases such as T2DM. Several fundamental cellular processes are governed by this crosstalk among the mitochondria, ER, and nucleus, which plays an essential role in the regulation of the cellular metabolic response to environmental cues. The blockage of this communication reduces mitochondrial activity [94,95] and increases the incidence rate of metabolic disease. Thus, the mitochondria govern intercommunication between cellular organelles, including ER and nucleus, via Ca^{2+} signaling to regulate metabolism.

Taken together, here, we propose that TFAM is involved in the regulation of Ca^{2+} flux via the mitochondria-ER interaction that can signal to the nucleus, which ultimately mitigates metabolic disorders.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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