CALCIUM AND TFAM CROSSTALK IN THE MITOCHONDRIAL LIFE CYCLE IN CARDIOMYOCYTES

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Abstract
Calcium (Ca^{2+}) links the electrical signals of the heart to the mechanical action of contraction in a process referred to as the cardiac excitation-contraction (EC) coupling, a process that consumes a large amount of adenosine triphosphate (ATP). The majority of ATP is produced in the mitochondria via oxidative phosphorylation (OXPHOS), which is linked to Ca^{2+} flux. The OXPHOS system is regulated by both the nuclear and mitochondrial genome, with mitochondrial transcription factor A (TFAM) being a major regulator of the latter. This mini review focuses on summarizing the limited literature implicating crosstalk between Ca^{2+} and TFAM in the adult cardiomyocyte throughout the mitochondrial life cycle: mitochondrial dynamics, biogenesis, and mitophagy. The goal of this review is to highlight gaps and fuel further investigation of the proposed Ca^{2+}-TFAM axis. This research area has high potential to propel the development of therapeutic strategies targeting cardiovascular diseases such as heart failure.
Keywords: mitochondria, calcium, TFAM, cardiomyocytes, heart

Resumen
El calcio (Ca^{2+}) vincula las señales eléctricas del corazón con la acción mecánica de la contracción en un proceso denominado acoplamiento excitación-contracción (EC) cardíaco, un proceso que consume una gran cantidad de trifosfato de adenosina (ATP). La mayor parte del ATP se produce en las mitocondrias mediante fosforilación oxidativa (OXPHOS), que está ligada al flujo de Ca^{2+}. El sistema OXPHOS está regulado tanto por el genoma nuclear como por el mitocondrial, siendo el factor de transcripción mitocondrial A (TFAM) un importante regulador de este último. Esta mini revisión se centra en resumir la literatura limitada que implica la interferencia entre Ca^{2+} y TFAM en el cardiomiocito adulto a lo largo del ciclo de vida mitocondrial: dinámica mitocondrial, biogénesis y mitofagia. El objetivo de esta revisión es resaltar las brechas e impulsar una mayor investigación del eje Ca^{2+}-TFAM propuesto. Esta área de investigación tiene un gran potencial para impulsar el desarrollo de estrategias terapéuticas dirigidas a enfermedades cardiovasculares como la insuficiencia cardíaca.
Palabras clave: mitocondrias, calcio, TFAM, cardiomiocitos, corazón

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Introduction

The adult mammalian heart is an energy-demanding organ that couples electrical signals to the mechanical contractions of the heart. Most of the energy used in the excitation-contraction (EC) comes from adenosine triphosphate (ATP) produced via oxidation phosphorylation (OXPHOS) performed in the mitochondria, membrane-bound sub-cellular organelles. It is estimated that each contraction utilizes 60-70% of generated ATP with the remaining used to fuel various ion pumps, especially the Ca^{2+} ATPase of the sarcoplasmic reticulum (SR) [1]. Thus, it is not surprising that mitochondria make up about 30% of the volume of cardiomyocytes, the building blocks of the heart contractile tissue. The expression of OXPHOS proteins is governed by both the nuclear and mitochondrial genomes – with the mitochondrial genome being regulated by mitochondrial transcription factor A (TFAM), a highly abundant DNA-binding protein that serves roles in both mitochondrial DNA (mtDNA) maintenance and transcription. While it is well accepted that Ca^{2+} stimulates OXPHOS through the citric acid cycle and various respiratory complexes [2], and that nuclear Ca^{2+} is a key regulator of gene expression, the role of Ca^{2+} in mitochondrial gene expression remains obscure.

Each heartbeat consists of a contraction initiated by a cardiac action potential with the activation of the voltage-gated sodium (Na^{+}) channels in phase 0. The resulting Na+ inward current induces a rapid depolarization of the cell membrane and consequent opening of the voltage-dependent L-type Ca^{2+} channels. This Ca^{2+} influx triggers the opening of ryanodine receptors (RyRs) located at the junctional sarcoplasmic reticulum (jSR), which causes the massive release of Ca^{2+} release referred to as calcium-induced calcium release (CICR). Once in the cytosol, Ca^{2+} binds myofilaments – i.e. troponin C – to induce contraction. Relaxation is initiated by the reuptake of Ca^{2+} by the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), and extrusion by the mitochondrial Na^{+}/ Ca^{2+} exchanger (NCLX), and the plasma membrane Ca^{2+} ATPase (PMCA).

Mitochondria also participate in the cellular uptake and extrusion of Ca^{2+} during cardiomyocyte contraction [3]. There are three distinct populations of mitochondria within the cardiomyocyte that have different structural [4] and biochemical properties [5]: subsarcolemmal (SSM), perinuclear (PNM), and interfibrillar (IFM). All three subtypes are subject to the mitochondrial life cycle with differing levels of OXPHOS and Ca^{2+} accumulation [5,6] The IFM make up majority of the population within the cardiomyocyte and are in close proximity (~50-100nm) to the jSR, which are privileged sites of rapid and potent local Ca^{2+} signaling events [7]. Voltage-dependent anion channel 1 (VDAC1) – located on the mitochondrial outer membrane (OMM) – is responsible for the Ca^{2+} influx in the intermembrane space (IMS), where it is selectively imported into the mitochondrial matrix through the mitochondrial Ca^{2+} uniporter (MCU). Mitochondrial matrix Ca^{2+} activates several dehydrogenases and enhances the electron transport chain (ETC) efficiency – and therefore ATP production – in a feedforward loop that balances energy supply and demand. This balancing act is known as cardiac excitation-contraction-bioenergetics coupling (ECB). Ca^{2+} can return to the IMS actively through the Na^{+}/Ca^{2+} (NCLX) or the H^{+}/Ca^{2+} exchangers, or passively under cellular stress through the opening of the mitochondrial permeability transition pore (mPTP).

This review examines the limited literature implicating crosstalk between Ca^{2+} and TFAM in the context of the energy metabolism of the cardiac tissue, an active area of research for the development of therapeutic strategies targeting cardiovascular diseases such as heart failure. In particular, we discuss the crosstalk between Ca^{2+} and TFAM in the regulation of mitochondrial dynamics, biogenesis, and mitophagy. The cardiomyocyte is a unique candidate to study this interplay due to its high reliance on Ca^{2+} for EC, and the unique nuances in the cardiac mitochondrial life cycle due to the high-energy demands of this post-mitotic tissue as well as the spatial limitations of the myofibril structure. Furthermore, compared to other tissues, cardiac tissue is exposed to several stressors, such as mechanical stress and ischemia/hypoxia.

TFAM and the Mitochondrial Genome

The mitochondrial genome is a circular DNA molecule of 16,659 base pair encoding 13 essential subunits of the ETC. The expression of mtDNA – maintained as a multicopy genome – is carried out by factors encoded in the nuclear genome. In addition, the 77 remaining subunits and all the assembly factors are also nuclear encoded. Thus, the biogenesis of the ETC and OXPHOS are governed by both genomes. MtDNA is compacted in discrete DNA-protein complexes known as mitochondrial nucleoids. Nucleoids have an average size of ~100 nm in diameter and typically contain a single mtDNA molecule. Super-resolution microscopy revealed populations of nucleoids with different characteristics. Larger, less compact nucleoids are actively used for transcription and genome replication while highly compacted ones are inactive and represent a form of protected mtDNA storage with a lower mutational load. This polyploidy nature of the mitochondrial genome allows for important features including heteroplasmy and clonal segregation within a single mitochondrion [8]. Mitochondrial heteroplasmy is the co-existence of different mtDNA genotypes, such as inherited polymorphisms or accumulated somatic mutations. These mutations are not all pathogenic, with defective genomes having to surpass a threshold of 60-80% to exhibit a clinical phenotype. Clonal expansion refers to the process behind how single deleterious mtDNA mutations can accumulate although this needs to be further studied.

The differential compaction of nucleoids is regulated by the ratio between TFAM and mtDNA. At high TFAM/mtDNA ratios, the nucleoid is fully compacted blocking both transcription and replication – similarly to nuclear heterochromatin [9]. While not yet well understood, it has been suggested that post-translational acetylation and phosphorylation of TFAM fine-tunes its ability to bind mtDNA, thus regulating mtDNA accessibility to replication and transcription factors [10]. This highlights the role of TFAM as a regulator of mtDNA heteroplasmy, and mtDNA heteroplasmy is at the core of age-associated heart failure [11]. TFAM is phosphorylated by AMP-activated protein kinase (AMPK) which impairs DNA binding and promotes its degradation by the AAA+ Lon protease [8]. AMPK is activated by phosphorylation by Ca^{2+}/calmodulin protein kinase kinase β (CaMKK β) which is regulated by Ca^{2+} [12]. This data is supportive of an indirect role for Ca^{2+} in regulating mtDNA expression via TFAM-mediated compaction.

TFAM also serves as the only known mitochondrial transcription factor. Transcription of the mitochondrial genome originates in the major non-coding region containing the H-strand (HSP) and L-strand (LSP) promoters. The initiation of mitochondrial transcription requires the association of the mitochondrial RNA polymerase (POLRMT) with TFAM and mitochondrial transcription factor B2 (TFB2M). At both the HSP and LSP, TFAM bound to DNA recruits POLRMT via its N-terminal extension and TFB2M modifies the structure of POLRMT to induce opening of the promoter [13].

TFAM in the Cardiomyocyte

Genetic ablation of TFAM in the cardiomyocytes at different developmental time points give rise to specific phenotypes. Heart-specific knockout of TFAM results in lethality at embryonic day (E) 15.5 and is marked by myocardial thinning and mtDNA depletion [14]; which is a shift from the global loss of TFAM, which results in embryonic lethality prior to E10.5 [15]. Skeletal/heart *Tfam* deletion at E13 results in dilated cardiomyopathy and atrioventricular conduction blocks resulting in death by 2-4 weeks postnatal [16]. Interestingly, shifting the timing of ablation of TFAM in cardiomyocytes to later in life requires a much longer time before mtDNA depletion and symptoms of cardiomyopathies are evident. The authors termed this process cardiac functional resistance [17]. Their study showed that it took months before there were distinguishable differences between control and TFAM-null animals in respects to cardiac ETC performance. Furthermore, a study utilizing a rescue mouse model expressing human TFAM in the heart showed that while there was a severe reduction in mtDNA transcription initiation, the mice were surprisingly healthy at 52 weeks due to a compensatory transcript stabilization mechanism that led to near-normal steady state level of transcripts [18]. These studies, along with the absence of studies on changes of TFAM expression during aging, highlight the importance and the need to further study the role of mitochondrial proteins

not just tissue specifically but also temporally, especially when it comes to diseases of aging such as cardiovascular diseases.

Furthermore, cardiomyocyte specific knockout (KO) of *Tfam* models mitochondrial cardiomyopathies by featuring increased uptake and diminished efflux of mitochondrial Ca^{2+} [16]. This study showed that cardiac mitochondria from *Tfam* KO mice take up Ca^{2+} at a twice the rate as controls and release Ca^{2+} at half the rate [19]. These changes are reflected by increased MCU complexes and decreased NCLX protein. The mechanism behind these changes in protein expression was not explored in this study, however, another study has established that diminished MCU degradation is responsible for the enhanced MCU activity in Tfam KO hearts due to a compensatory mechanism mediated by Complex I dysfunction [20]. This increased mitochondrial Ca^{2+} maintains respiratory rates despite compromised OXPHOS, illustrating that Ca^{2+} accumulation is capable of rescuing deficits in energy synthesis capacity *in vitro* [19]. However, it should be noted that both too high and too low concentrations of Ca^{2+} can negatively impact OXPHOS, RPS production, and/or lead to apoptosis [21]. Also, it is interesting to note that alterations in mitochondrial function and cytosolic Ca^{2+} induced by hyperglycemia are restored by overexpression of TFAM in neonatal cardiomyocytes [22].

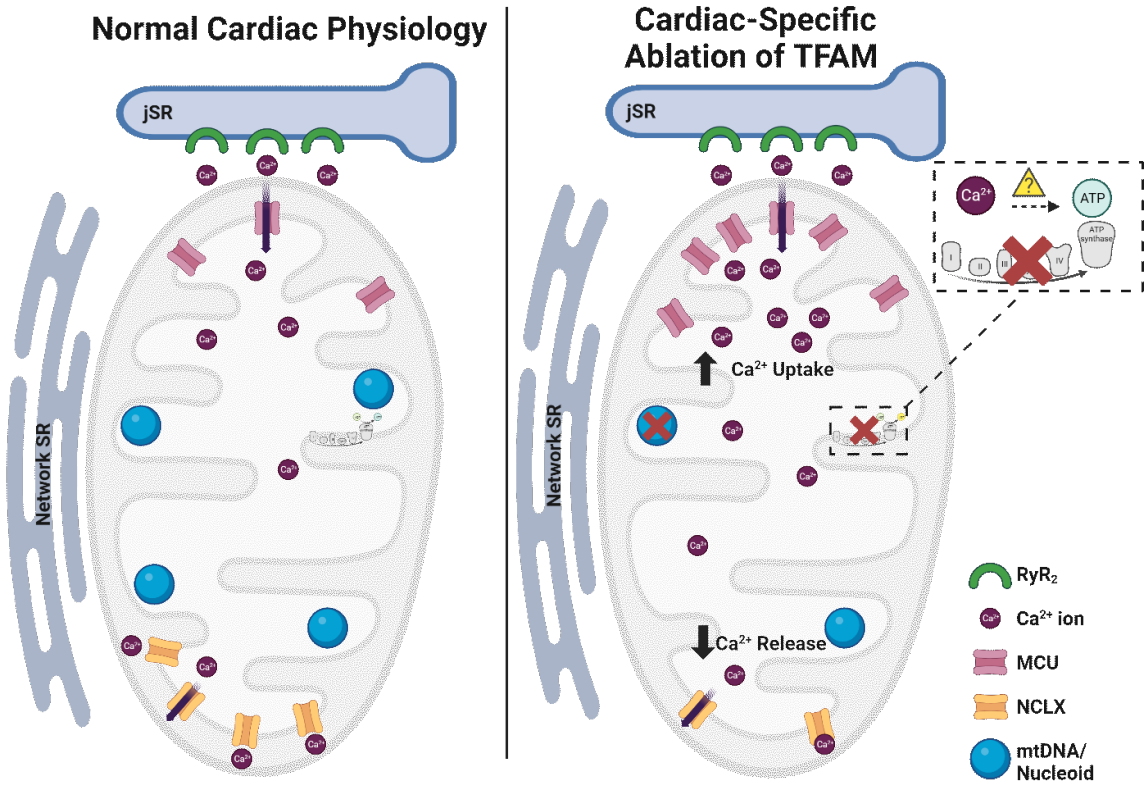

Figure 1. Schematic of cardiac-specific TFAM ablation and its disruption of mtCa^{2+} flux. In normal cardiac physiology, contraction is caused by Ca^{2+} release from ryanodine 2 receptors (RyR_2) in the junctional sarcoplasmic reticulum (jSR). While the majority of this Ca^{2+} diffuses rapidly into the cytosol where it binds myofilaments to induce contraction, mitochondria are also able to uptake of Ca^{2+}. Voltage-dependent anion channel 1(VDAC1) (not depicted) on the outer membrane allows entry of Ca^{2+} into the mitochondrial intermembrane space (IMS) where it is selectively imported in mitochondrial matrix through mitochondrial calcium uniporter (MCU). Ca^{2+} can return to the IMS actively through the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) or the H^+/Ca^{2+} exchangers. However, a mouse model with a cardiomyocyte specific knockout (KO) of *Tfam*, results in the expected decrease of mitochondrial DNA (mtDNA) and compromised oxidative phosphorylation (OXPHOS), also shows an increased expression of MCU complexes and decreased expression of NCLX. This results in Ca^{2+} uptake occurring at a twice the rate and release of Ca^{2+} at half the rate of controls. This increased mitochondrial Ca^{2+} *in vitro* rescues respiratory rates despite compromised OXPHOS, although the underlying mechanism for this phenomenon is unknown. Figure created summarize findings from [19] with BioRender.com.

The Ca^{2+} and TFAM Connection in Mitochondrial Dynamics

Mitochondrial dynamics, the dynamic interplay between mitochondrial fission and fusion contributes to the regulation of both mitochondrial biogenesis and mitophagy. While we have known that both fission and fusion related proteins are highly expressed in adult cardiomyocytes, it was assumed – until very recently – that cardiac mitochondrial dynamics were fairly static. These assumptions were due to the structurally and spatially restrictive nature of cardiomyocytes that was thought to hinder mitochondrial motility; as well as the inability to observe these processes in adult cardiomyocytes.

Mitochondrial fission is regulated by both dynamin-related protein 1 (DRP1) and intracellular Ca^{2+} [23]. Fission plays roles in both removing damaged mitochondrial components and mitochondrial biogenesis, including the structure and dispersion of nucleoids [24]. Furthermore, nucleoids and fission are spatially linked at endoplasmic reticulum (ER)-mitochondria contact sites (ERMCs) which couple mtDNA synthesis to mitochondrial division [25] and knockdown of TFAM causes nucleoid aggregation and a reorganization of nucleoids at mitochondria-ER contact sites [26]. In 2021, it was established in COS-7 cells and primary mouse cardiomyocytes that distinct fission signatures predict whether daughter mitochondria are destined for degradation or biogenesis [27]. Division at the periphery was associated with mitophagy, whereas division at the midzone was associated with biogenesis. Both processes are mediated by DRP1, ER contact and actin-mediated pre-constriction but differ by adaptors with mitochondrial fission factor (MFF) governing midzone fission and peripheral fission requiring lysosomal contact and regulated by the OMM protein, FIS1. Constriction of the inner mitochondrial membrane (IMM) is mechanistically not well understood, but it has been shown it could be dictated by intra-mitochondrial influx of Ca^{2+} [28]. Thus, mitochondrial Ca^{2+} regulation can regulate fission dynamics, that in turn can regulate the distribution of TFAM compacted nucleoids. However, coupling of mtDNA replication with nucleoid distribution and fission to the cardiac specific SR, has yet to be examined.

Mitochondrial fusion involves the fusion of outer mitochondrial membranes mediated by the proteins mitofusin 1 and 2 (MFN1 and MFN2), and that of inner mitochondrial membranes mediated by optic atrophy 1 (OPA1), with the latter process being less understood. Mitochondrial fusion promotes quality control and facilitate the exchange of genetic material and functional components of mitochondria. Eisner *et al.* used mitochondrial imaging of whole rat hears expressing a mitochondrial matrix photoactivable GFP to to demonstrate that robust fusion activity occurs in cardiomyocytes and depends on Ca^{2+} oscillations and contraction [29]. This study also showed that this activity decayed rapidly in culture likely due to the loss of Ca^{2+} oscillations and contractile activity. Lastly, they further noted that weakened cardiac contractility *in vivo* in alcoholic animals was also associated with depressed mitochondrial fusion. In support of a connection between mitochondrial fusion, nucleoid distribution and Ca^{2+}, studies have shown that OPA1 variants that include exon 4b are required for maintenance of normal TFAM/nucleoid distribution independent of mitochondrial fusion [30, 31], that OPA1 modulates Ca^{2+} uptake through ER-mitochondrial coupling and that its loss disturbs Ca^{2+} homeostasis [32].

It should also be noted that mitochondria within cardiomyocytes have been observed to exchange contents via “kissing” and nanotunnel formation [33]. These nanotunnels have been shown to form in immobilized mitochondria, as well as in mitochondria where fission and fusion processes fail. In addition, disruption of Ca^{2+} dynamics such as RyR dysfunction has been associated with a greater abundance of the formation of these mitochondrial nanotunnels in cardiomyocytes [34]. Nanotunnels are hypothesized to transport ions such as Ca^{2+}, small proteins, RNA and free mtDNA between mitochondria [33]. In general, it is believed that nucleoid-packaged mtDNA are too large to pass through them with the exception of cardiomyocyte nanotunnels, which have been observed to have diameters large enough to support the transport of the TFAM-packaged mtDNA [33]. However, whether they transport Ca^{2+} and nucleoids has yet to be determined. In addition, it has been proposed that these larger nanotunnel-like structures in cardiomyocytes are in the process of expanding to form cristae-bearing tubular mitochondria [35].

The Ca^{2+} and TFAM Connection in Mitochondrial Biogenesis

Like OXPHOS**,** mitochondrial biogenesis is governed by both genomes. Nuclear-encoded factors, including transcription factors, coactivators, and signaling molecules, regulate the expression of mitochondrial genes and coordinate mitochondrial biogenesis. Emerging evidence suggests that Ca^{2+} signaling modulates mitochondrial biogenesis through direct and indirect mechanisms by affecting these factors.

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a master regulator of both mitochondrial biogenesis and function. PGC-1α is induced by various signaling pathways, including Ca^{2+} signaling. Ca^{2+} signaling can activate PGC-1α through Ca^{2+}/calmodulin-dependent protein kinases (CaMKs) and Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK). Upon activation, CaMKs phosphorylate and activate PGC-1α, promoting its nuclear translocation and subsequent activation of target genes involved in mitochondrial biogenesis. Ca^{2+} signaling also enhances the interaction between PGC-1α and its transcriptional partners, such as nuclear respiratory factors (NRFs) and estrogen-related receptors (ERRs), further amplifying mitochondrial biogenesis.

NRF-1 and NRF-2 promote transcription of nuclear-encoded mitochondrial genes, including *Tfam* [36]. A study has also shown that TFAM signals to the nucleus by Ca^{2+}/CaMKKB pathway to induce PGC-1α and PPARβ [37], demonstrating that this Ca^{2+} and TFAM crosstalk is bidirectional. This study also showed that TFAM may mediate mild uncoupling of the mitochondrial membrane potential ($\Delta\Psi_m$) which has been shown to regulate Ca^{2+} uptake. ERRs, specifically ERRα, ERRβ, and ERRγ, are orphan nuclear receptors that cooperate with PGC-1α to drive mitochondrial biogenesis. ERRα regulates mitochondrial biogenesis, mitophagy and mitochondrial turnover by directly inducing *TFAM*, *TFB2M*, *NRF1*, *MFN2*, and *SIRT3* gene expression [38,39] TFAM overexpression has also been shown to increase the levels of pAMPK leading to enhanced PGC-1α and PPARβ, establishing a potential positive feedback loop.

The Potential for Ca^{2+} and TFAM Connection in Mitophagy

Mitophagy, the selective removal of damaged mitochondria, is a critical quality control mechanism in the mitochondrial life cycle [40]. We know that excessive mitophagy coupled with altered mitochondrial Ca^{2+} uptake is implicated in various diseases including cardiovascular disease. While there are no direct links reported in the literature between TFAM and mitophagy, knockdown of TFAM has been implicated in both reducing and promoting autophagy depending on the cell line [41,42]. Furthermore, TFAM heterozygous knockdown mice (TFAM+/-) exhibit disruption of mtDNA stability that leads to cytosolic release of mtDNA and an innate immune system response which can trigger mitophagy [43]. Thus, highlighting the need for understanding a possible role of TFAM in mitophagy specifically in cardiomyocytes.

The role of Ca^{2+} in mitophagy is still somewhat controversial and it is partially summarized in Figure 2 [44]. Both PINK1 and Parkin, activators of the mitochondrial quality control system, target the outer mitochondrial membrane Rho GTPase 1 Miro1, which controls mitochondrial shape transition in a Ca^{2+}-dependent manner. Miro1 activity is a prerequisite for the initiation of mitophagy [45]. In addition, mutations of Miro1 have been demonstrated to lead to reduced ER-mitochondria tethering coupled to aberrant Ca^{2+} signaling, which induced mitophagy. Furthermore, PINK1 phosphorylates leucine zipper and EF-hand containing transmembrane protein 1 (LETM1), a mitochondrial inner membrane protein that mediates mitochondrial proton-dependent Ca^{2+} exchange. In neurons, where mitophagy is largely studied, the absence of PINK1 leads to an increased mitochondrial Ca^{2+} sensitivity that precedes neuronal decay. These findings highlight an interesting phenomenon that is yet to be examined in cardiomyocytes. Both PINK1 and Parkin are present at the ER-mitochondria interface where Ca^{2+} communication is concentrated. PINK1 and Parkin also interact with other proteins localized at these sites – including VDAC and MFN2 – indicating a connection between Ca^{2+} signaling and/or mitophagy is plausible. Finally, the overexpression of Parkin has been shown to enhance the structure and function of the ER-mitochondria connection. Whether this enhancement extends to SR-mitochondria connections would be an interesting area of study in cardiomyocytes.

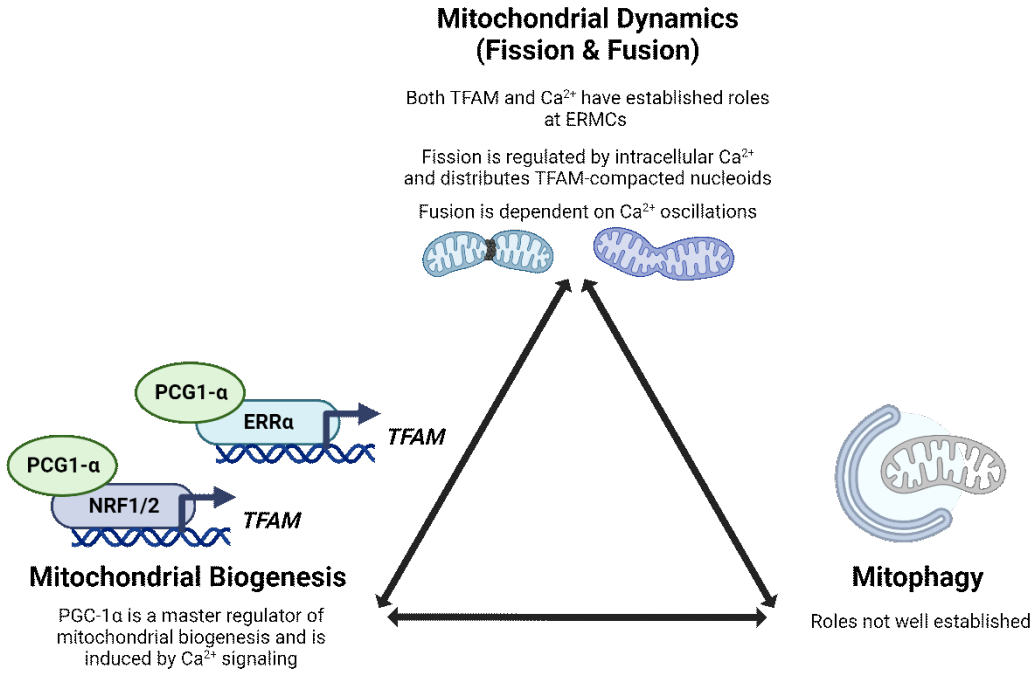

Figure 2. Summarized Ca^{2+}-TFAM crosstalk in the mitochondrial life cycle.
The mitochondrial life cycle comprises mitochondrial dynamics (fission and fusion), mitochondrial biogenesis and mitophagy. Both mitochondrial transcription factor A (TFAM) and Ca^{2+} have established roles at ER-mitochondria contact sites (ERMCs) which are known sites for both mtDNA replication and fission. Furthermore, fission is regulated by intracellular Ca^{2+} and responsible for the distribution of TFAM-compacted nucleoids. Fusion is also a Ca^{2+}-dependent process. In mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a master regulator and induced by Ca^{2+} signaling. PGC-1α promotes transcription of *Tfam*. Ca^{2+} signaling also enhances the interaction between PGC-1α and its transcriptional partners nuclear respiratory factors (NRFs) and estrogen-related receptors (ERRs) which also promote transcription of *Tfam*. As for mitophagy, roles for neither Ca^{2+} nor TFAM are well established. Figure created with BioRender.com.

Conclusions

This review has provided an overview of the interplay between Ca^{2+} and TFAM in cardiomyocytes at different stages of the mitochondrial life cycle: biogenesis, mitophagy and network dynamics. A direct connection between TFAM and Ca^{2+} in cardiomyocyte has been shown as the genetic ablation of TFAM leads to an increased uptake and diminished efflux of mitochondrial Ca^{2+}. The literature also supports the idea of a feedforward loop between Ca^{2+} and TFAM in the context of mitochondrial biogenesis and a connection between the two in mitochondrial dynamics. However, the molecular players involved in such processes also share several other roles in mitochondrial life cycle regulation, complicating the design of targeted experiments. For example, Parkin-independent mitophagy requires the mitochondrial fission protein Drp1 to maintain integrity of the mammalian heart [46]. It is especially important to understand these dualistic roles in the context of adult post-mitotic cardiomyocytes, to understand how this may contribute to cardiovascular pathologies. In general, we are far from understanding the intricacies of TFAM and the Ca^{2+}-TFAM axis in regulating mtDNA heteroplasmy in the context of cardiovascular diseases.

In conclusion, the different stages of mitochondrial life cycle play crucial roles in both the physiological heart function, and its adaptive response to extenuating workload, ischemia, oxidative stress and other stressors. Therefore, the impairment of either mitochondrial dynamics, biogenesis, or mitophagy are associated with the development and progression of various cardiovascular diseases. Thus, our understanding of basic cardiac function – and therapeutic interventions – would immensely benefit from a better understanding of the Ca^{2+}-TFAM signaling.

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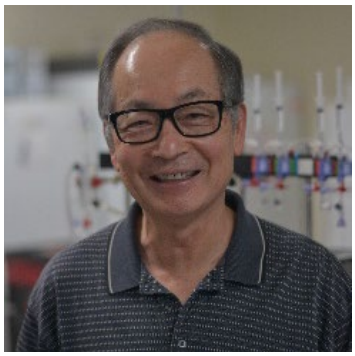

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