Review Article
Regulation of mitochondrial ATP synthase in cardiac pathophysiology

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Abstract: Mitochondrial function is paramount to energy homeostasis, metabolism, signaling, and apoptosis in cells. Mitochondrial complex V (ATP synthase), a molecular motor, is the ultimate ATP generator and a key determinant of mitochondrial function. ATP synthase catalyzes the final coupling step of oxidative phosphorylation to supply energy in the form of ATP. Alterations at this step will crucially impact mitochondrial respiration and hence cardiac performance. It is well established that cardiac contractility is strongly dependent on the mitochondria, and that myocardial ATP depletion is a key feature of heart failure. ATP synthase dysfunction can cause and exacerbate human diseases, such as cardiomyopathy and heart failure. While ATP synthase has been extensively studied, essential questions related to how the regulation of ATP synthase determines energy metabolism in the heart linger and therapies targeting this important mechanism remain scarce. This review will visit the main findings, identify unsolved issues and provide insights into potential future perspectives related to the regulation of ATP synthase and cardiac pathophysiology.

Keywords: ATP synthase, mitochondria, cardiac hypertrophy, heart failure, energy metabolism, mPTP

Introduction

Mitochondrial complex V or ATP synthase is an enzyme complex that works as a molecular machine to generate and hydrolyze ATP in cells in the last step of the mitochondrial respiratory process. Therefore, ATP synthase plays pivotal roles not only in maintaining the cellular energy state, but also in determining mitochondrial respiratory function. Cardiac contraction and relaxation are energy demanding processes that depend on mitochondrial function and efficient ATP production/reservation. Dysregulation of ATP synthase activity should have major impacts on mitochondrial respiration and hence cardiac performance. Mitochondrial energy disturbances are involved in cardiac pathological development [1]. For example, myocardial ATP depletion is a key issue of heart failure [1-3]. Therefore, further research on the regulation of the mitochondrial ATP synthase in the heart may help discover novel therapeutic strategies for the treatment of cardiac disorders. This review will discuss the current knowledge of ATP synthase regulation in the heart, the potential challenges in the field, and the potential perspectives on the translational potential of the related research.

ATP synthase: a molecular machine that makes ATP

ATP synthase, also known as F_1F_0-ATP synthase or complex V, is the key energy generator for most life forms on earth. This large, mitochondrial protein complex is bound to the inner mitochondrial membrane along with the other respiratory chain complexes I-IV. The enzyme functions through a reversible rotary complex, whereby the direction of its rotation determines the synthesis or hydrolysis of ATP. In order to generate ATP from ADP and Pi, ATP synthase rotates using the driving force of an electrochemical potential built up in the intermembrane space by the I-IV respiratory chain complexes (see review [4, 5]. Conversely, ATP synthase can spin in the reverse direction and hydrolyze ATP to pump protons back to the intermembrane space to maintain membrane potential. ATP synthase consists of two distinct subcomplexes with complementary functions. The Fo complex contains transmembrane sub-
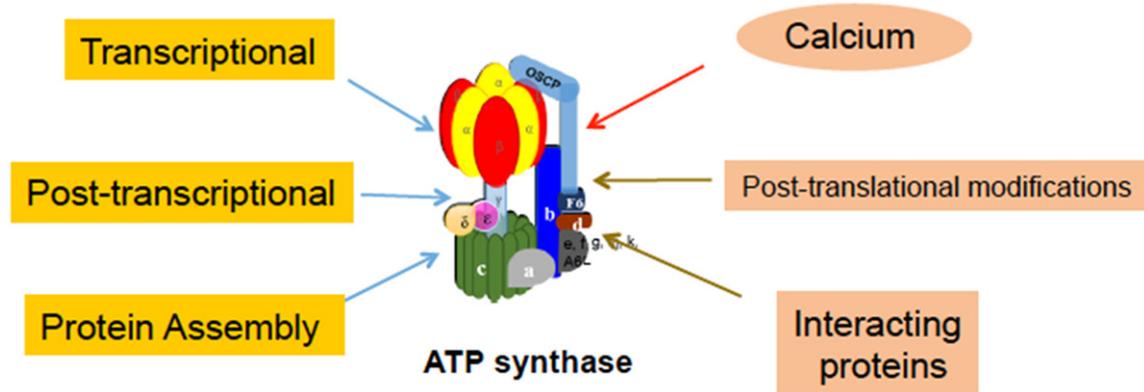
Figure 1. Summary of ATP synthase activity regulation. The steady state of mitochondrial ATP synthase activity is regulated at the transcriptional, post-transcriptional and protein assembly levels and the dynamic state of mitochondrial ATP synthase activity is regulated by calcium transient, post-translational modifications and interacting proteins.
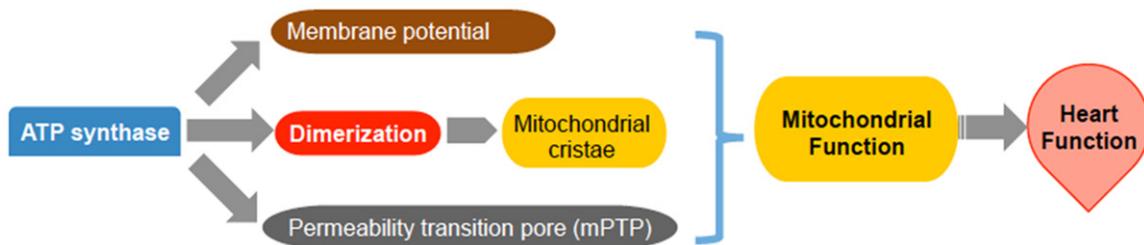
Figure 2. The roles of ATP synthase in mitochondrial function. The mitochondrial ATP synthase activity plays a key role in mitochondrial function and cardiac function in determining membrane potential, mitochondrial cristae and the opening of the mitochondrial permeability transition pore (mPTP).

units that transport protons from the intermembrane space and the F1 is a peripheral complex in the matrix, which catalyzes nucleotide binding for ATP synthesis [6-9]. F_o and F_1 are connected through two stalk-like subunits: a central rotor shaft and a peripheral stator [6]. In the mammalian mitochondrial enzyme, F_1 is composed of three copies each of subunits α and β, and one each of subunits γ, δ and ε. F_o consists of a subunit c ring (comprising 12 copies) and one copy each of subunits a, b, d, h (F_6) and the O subunit or oligomycin sensitivity conferring protein (OSCP). A number of additional subunits (e, f, g, i/j, k and A6L) are associated with F_o, although their precise locations within the complex remain unknown [10-13]. Protons accumulated in the intermembrane space are driven through a channel in F_o, which causes rotation of the c-ring along with the attached central stalk. Subsequently, rotation of subunit γ, within the F_1-$α_3β_3$ hexamer provides energy for ATP synthesis at the catalytic sites (located in each of the three β subunits, at the interface

with an adjacent α subunit) [14]. The rotation of the F_1 hexamer ($α_3β_3$) may enable the Interconversion of the states relative to the γ-subunit [6].

Despite the accumulation of this detailed knowledge of ATP synthase constituents in the past two decades, the roles of ATP synthase associated proteins in this crucial enzyme complex remain understudied, especially in the *in vivo* context. So far, most of the genetic investigations related to in the vitro function of ATP synthase have been conducted on either cultured cells or yeast. The exciting development in molecular genetics that enable genetic manipulations to be done with relative ease will open new doors for the further *in vivo* study of the function and regulation of this most important enzyme complex in our body.

The regulation of ATP synthase activity

The in-depth understanding of how the ATP synthase works to generate and hydrolyze ATP is

well known, but the underlying mechanism of how ATP synthase is regulated remains obscure. Current literatures propose multi-levels of regulating mechanisms for ATP synthase activity, which primarily rely on elements directly involved in its operation. Among these elements are ADP, Mg^{2+}, Pi, ATP, and others, such as anions [15-17]. The heart is known to be an energy-demanding organ for contraction/relaxation and ion transport [18], but the mechanisms that enable it to alter rapidly the ATP level to meet the fluctuating demand remain unclear. In general, the mitochondrial ATP synthase is regulated to maintain its steady and dynamic states of capacity.

Steady state regulation

Given that the ATP synthase plays such a pivotal role in cellular function, it is essential for maintaining the constitutive expression of key components of this enzyme complex. Based on current literatures, it appears that transcriptional, post-transcriptional, and protein assembling regulations determine the steady state of the ATP synthase activity (**Figure 1**).

Transcriptional regulation: Transcriptional regulation of metabolisms is essential in controlling the rate of metabolism in response to various physiological and pathological cues. Transcription factors, such as many nuclear receptors, are among the key transcriptional regulators of metabolic pathways [19]. Because genes for enzymes of oxidative phosphorylation are thought to be housekeeping genes that are transcribed constitutively [20], the transcriptional regulation of the component proteins of ATP synthase has been limited. In general, changes of ATP synthase content or activity appear to occur preferentially at the protein levels. However, mutations that mostly lead to the deficiency of the enzyme have been identified on genes encoding ATP synthase component proteins. Clinical cases with nuclear genetic defects of mitochondrial ATP synthase, such as the mitochondrial DNA *ATP6* and the nuclear *ATP12* genes [21, 22], have been reported. They are characterized by early onset, lactic acidosis, 3-methylglutaconic aciduria, hypertrophic cardiomyopathy, and encephalopathy, followed by premature death [23]. On the other hand, it appears that the transcripts of ATP synthase components could be regulated by common transcription factors, such as peroxisome

proliferator activator receptor δ (PPARδ) [24, 25] and estrogen related receptors (ERRs) [26]. Such regulation could lead to the co-activation of peroxisome proliferator activator receptor γ [26] as part of the overall metabolic responses under different circumstances. Intuitively, ATP synthase transcripts are expressed at different tissue-specific levels with higher levels found in skeletal muscle and heart and lower levels in other tissues [27]. Specifically, *in vitro* studies have demonstrated that the transcriptional expression of ATP synthase components is controlled by various transcriptional regulation factors. For example, ATP factor 1 (ATPF1), which is present in human HeLa nuclei, plays a critical role in transcriptional activation of the α subunit of the ATP synthase [28]. The same group further illustrated that upstream stimulatory factor 2 (USF2) [29-31] and the transcription factor Yin Yang 1 (YY1) promotes transcription expression of the α subunit [32]. To further exemplify, it has been shown that hypoxia suppresses the transcript expression of the subunit e of ATP synthase. Therefore, ATP synthase could also be regulated at the transcriptional level by oxygen availability [33].

Because of the general lack of *in vivo* information, the significance of transcriptional regulation of the mitochondrial ATP synthase on the development of myocardial pathophysiology is not clear. However, the human cases of ATP synthase deficiency have clearly manifested how crucial it is to maintain an optimal level of ATP synthase in the body, especially for preserving the normal function of the heart.

Post-transcriptional regulation: The ATP synthase is also regulated at the post-transcriptional level by controlling translation of the enzyme complex. For instance, the expression of its catalytic subunit (β subunit) is stringently controlled at post-transcriptional levels. Micro-RNA plays an important role in regulating the translation of the β subunit. Willers IM *et al* showed that miR-127-5p represses the β subunit translation by inhibiting the 3'UTR of the β subunit mRNA of human ATP synthase [34].

The regulation of protein assembly: The assembly of F_1 hexamer structure requires two specialized chaperones, Atp11p and Atp12p in yeast [35], which bind transiently to the α and β subunits. In the absence of Atp11p and Atp12p, the hexamer is not formed, and the α and β

subunits precipitate as large insoluble aggregates [35-38]. This appears to be the case in humans too [39]. Mutants lacking the α and β subunits of F (1), or the Atp11p and Atp12p chaperones that promote F (1) assembly, have normal levels of the bicistronic ATP8/ATP6 mRNAs, but fail to synthesize Atp6p and Atp8p [40]. Another recent study showed that the INA complex facilitates assembly of the peripheral stalk of the mitochondrial F_1F_o-ATP synthase [41]. Additionally, it has been shown that OSCP plays a key role in the biogenesis of ATP synthase [42]. It is likely that p53 interacts with OSCP to facilitate the assembly and stabilization the ATP synthase complex [43].

Dynamic regulation

Because changes in cellular energy demand occur instantly and fluctuate rapidly, flux through mitochondrial ATP synthase must also change to maintain cellular ATP levels. Direct regulations at the level of ATP synthase appear to occur in mitochondria of these cells. ATP synthase activity increases in rat cardiomyocytes subjected to high-energy demand (beating, positive inotropic substances) and decreases in anoxic cells [44-46]. Several dynamic regulatory elements have been shown to act at the level of the ATP synthase (**Figure 1**).

Regulation of ATP synthase activity by mitochondrial calcium (Ca²⁺): Mitochondrial Ca^{2+} transients occur during the contractile/relaxation cycle and are translated into overall rise in mitochondrial ATP production to keep pace with the functional demand [47]. Therefore, mitochondrial Ca^{2+} plays crucial roles in the regulation of the ATP synthase activity. However, the molecular mechanisms underpinning the direct regulation of calcium on the ATP synthase remain obscure. Early studies based on purified protein showed Ca^{2+} might regulate the ATP synthase via a protein named calcium binding ATPase inhibitor (CaBI) [15, 16, 24]. CaBI is reported to be a 6.3 kD protein, which interacts with ATP synthase in a Ca^{2+} dependent manner [48, 49]. *In vitro* protein treatment of purified CaBI on extracted ATP synthase promotes ATP synthesis and inhibits ATP hydrolysis [49]. Nevertheless, as of today, very little is known as no specific gene that encodes for this elusive protein has been identified. Recently, a report showed that the β subunit of the ATP synthase binds Ca^{2+}, but with unknown effects [50]. In addition, Protein kinase Cδ, which is a Ca^{2+} sig-

naling protein, can regulate ATP synthase by binding to the d subunit of the F_o sector [51, 52]. It has also been reported that S100A1 is an F_1 interacting protein in the mitochondria and promotes ATP synthesis in a Ca^{2+} -dependent manner [53]. Despite these advancements, our understanding of how mitochondrial Ca^{2+} directly regulates the ATP synthase activity remain poor, due partly to the technical difficulties involved in measuring acute alteration of ATP contents in different cellular compartments, especially in the mitochondria. Recent developments using fluorescent markers for the real time measurement of mitochondrial Ca^{2+} in cultured cells [54] provide new tools for the field to gain better insight toward solving at least part of the puzzle.

Regulation of ATP synthase activity by post-translational modifications: Not surprisingly, posttranslational modifications (PTM) of ATP synthase play important roles in the regulation of ATP synthase activity. Evidence of direct regulations by post-translational modifications on key subunits of ATP synthase has been surprisingly limited [55-58]. However, more research has been emerging, including reports on several post-translational modifications on various subunits of the ATP synthase complex (see review [59]). Human ATP synthase β is phosphorylated at multiple sites and shows abnormal phosphorylation at specific sites in insulin-resistant muscle [57]. Further, the β-subunit of the ATP synthase is phosphorylated following myocardial preconditioning in rabbit myocytes [60]. In a study using a ^{32}P γ subunit labeling strategy, Hopper *et al* observed that the γ subunit of ATP synthase subunit was phosphorylated [61]. Additionally, Ko *et al* employed Phospho-tyrosine antibodies to confirm that the platelet-derived growth factor (PDGF) induced phosphorylation of the δ subunit [62]. This group also used ^{32}P labeling to show that the δ subunit could be differentially phosphorylated *in vitro* by mitochondrial extracts that had been isolated from either untreated NIH3T3 cells or from PDGF-treated NIH3T3 cells. However, further studies are required to understand how phosphorylation of a specific subunit of ATP synthase alters its function.

Using a proteomic approach, Wang *et al* uncovered several oxidative stress-related protein modifications occurring on ATP synthase in failing dyssynchronous hearts, which can be corrected by the cardiac resynchronization therapy

Table 1. Interacting proteins of the mitochondrial ATP Synthase

	Interactions	Functions	Citations
IF1	F1α, F1β	ATP synthase dimer formation	[100, 117]
Cyclophilin D	OSCP, subunit d, and subunit b	mPTP	[88, 118, 119]
Bcl-xL	F1α, F1β	Membrane potential, mPTP and apoptosis	[91, 120]
p53	OSCP	Apoptosis, mPTP	[43, 121, 122]
S100A1	F1α, F1β	Increase of ATP synthase activity	[53]
Factor B	F1α, OSCP	Component for ATP synthase complex formation	[82, 123]
Strap	F1β	Modulator for cellular energy metabolism	[124]
PKCδ	subunit d	Inhibited ATP synthase activity	[86, 87]

Shown is a list of well-characterized interacting proteins of the mitochondrial ATP synthase along with information of the corresponding interacting subunits of ATP synthase and the related functions.

(CRT), a clinically effective treatment for failing dyssynchronous hearts [63]. Multiple oxidative posttranslational modifications occur at a selective Cysteine in ATP synthase α subunit, which may act as a redox sensor modulating ATP synthase function [63]. The role of oxidative posttranslational modifications in the regulation of the ATP synthase complex is most frequently discussed in the context of heart failure and its possible clinical treatment [64]. Most recently, epigenetic regulation of ATP synthase has also been uncovered. Several subunits of the ATP synthase complex contain lysine modifications, including methylation and acetylation. For example, SIRT3 deacetylates ATP synthase F_1 complex proteins in response to nutrient- and exercise-induced stress [65]. However, it remains unknown if these posttranslational modifications also occur in cardiomyocytes and the *in vivo* context. Further investigations are needed to explore the translational potential of targeting the post-translational modification of ATP synthase.

Regulation of ATP synthase activity by its interacting proteins: Some proteins that are associated with ATP synthase, but not considered to be subunits, are also often involved in the regulation of mitochondrial ATP synthase. **Table 1** has summarized the ATP synthase interacting proteins in the literatures and their potential function in controlling the enzyme's activity.

About six decades ago, inhibitor factor 1 (IF1) was identified to be the first nuclear-encoded ATP-synthase interacting protein [66-68]. IF1 is an evolutionarily well conserved mitochondrial protein that interacts with the F_1 sector of ATP synthase and is not considered a subunit of the mitochondrial ATP synthase [69, 70]. Numerous studies confirmed that IF1 inhibits the ATP hydrolysis activity of the mitochondrial ATP-synthase [66, 69-71]. Interestingly, IF1 is activated under acidic conditions, such as in myocardial ischemia [72, 73]. ATP hydrolysis occurs when the electrochemical proton gradient across the mitochondrial inner membrance is lost (e.g., during hypoxic/ischemic conditions), and the enzyme reverses in an attempt to restore mitochondrial membrane potential [74, 75]. Therefore, IF1 is a potential drug target for enhancing ATP reserves in the heart [69, 70]. In fact, preclinical assessments on IF-1 mimetic compounds have shown promising results in animal studies [73, 76].

Most of the early knowledge of IF1 is based on studies on bovine heart mitochondria and has shown that IF1 can respond rapidly to the energy state of the mitochondrial membrane [18, 77]. IF1 interacts with the ATP synthase in mitochondria of many species, including the rat, even though its inhibitory function on ATP hydrolysis at least in the heart seems less effective in small animals than in large animals [78-80]. This conclusion appears to reduce the enthusiasm for studying IF1 in genetically manipulated mouse models. This view may hold some true at least in terms of IF1's cardiac role. A recent study on an IF1 knockout mouse model showed no basal phenotype, although ATP hydrolysis was elevated at least in mitochondrial samples extracted from liver [81]. Whether ATPase activity is affected in the IF1 knockout heart remains unknown. As stated previously, IF1 is activated under acidic conditions, so further studies are warranted to test if the loss of IF1 in mice under myocardial ischemia would lose the capacity to prevent accelerated ATP depletion.

Other ATP synthase interacting proteins have been identified: factor B [82-85], which is essential for ATP synthesis and involved in the regulation of ATP synthase oligomerization; CaBI [48], which may upregulate the enzyme activity in response to higher levels of cytoplasmic Ca^{2+}, and finally S100A1 [53], which improves catalytic efficiency in cardiac muscle. In recent years, a few more proteins have been shown to interact with and regulate ATP synthase. Nguyen *et al* showed that Protein kinase Cδ interacts with the d subunit of the F_o sector and inhibits ATPase activity [52, 86, 87]. It has also been reported that cyclophilin D, a member of the cyclophilin family of chaperones, can constitutively bind ATP synthase, thus slowing ATP synthesis and hydrolysis rates through interaction with the lateral stalk [88-90]. Additionally, recent studies have shown that the Bcl II family member, Bcl-xL, interacts with ATP synthase to inhibit ATPase activity in neurons [91]. Surprisingly, it was discovered that the tumor suppressor protein p53 is localized to the mitochondria and interacts with ATP synthase by binding to OSCP [43]. Furthermore, Stress-responsive activator of p300 (Strap), a p53 cofactor, interacts with the β subunit of ATP synthase to inhibit ATP synthase activity with similar potency as oligomycin and to induce apoptosis [92].

It has long been suggested that IF1 is the only naturally occurring, endogenous protein that interacts with the mitochondrial ATP synthase. However, it becomes clear that many more endogenous proteins must be involved in the regulation of mitochondrial ATP synthase. Further in-depth investigations are necessary to explore the significance of these newly emerging interacting proteins in regulating the enzyme activity of ATP synthase *in vivo*.

The role of ATP synthase in mitochondrial function

The mitochondrial ATP synthase certainly does not serve its sole function as an energy generator. By reversing ATP hydrolysis activity, ATP synthase plays a vital role in maintaining mitochondrial membrane potential [93] (**Figure 2**). IF1 is the first identified natural protein that prevents excess hydrolysis of ATP [69, 70]. It has also been reported that ATP synthase is involved in mitochondrial protein import [94] and the mobilization of cytochrome c during apoptosis [95]. Further evidence implicates that ATP synthase

dimerization, which is facilitated by IF1, plays an important role in forming mitochondrial cristae and the F_o c-ring itself or the dimerized ATP synthase may form the permeability transition pore (mPTP) (**Figure 2**). All of these should be crucial in maintaining mitochondrial function.

ATP synthase is suggested to have a role in crista morphogenesis. A recent study suggests that ATP synthase contributes to the optimal supramolecular organization of the respiratory chain [96], and even the density of cristae structure [97, 98]. ATP synthase occupancy rises correspondingly with the cellular demand for OXPHOS. Mitochondrial ATP synthases cluster as discrete domains that reorganize with the cellular demand for oxidative phosphorylation [99] and play a key role in cristae morphogenesis [98]. Other than inhibiting ATPase activity, IF1 may also regulate the oligomeric state of ATP synthase by facilitating the dimerization of ATP synthase via a molecular link between two F_1 domains [100]. IF1 limits the apoptotic-signalling cascade by preventing mitochondrial remodeling and preserves cristae structure to limit apoptotic cell death signaling [101].

However, the role of IF1 in promoting the dimerization of ATP synthase and mitochondrial remodeling is still under debate. Studies on mitochondrial extracted from bovine heart showed that the ATP synthase dimer is a stable inactive structure and its formation is not mediated by IF1 binding [102]. A cell culture study in human HeLa cells could not confirm that IF1 overexpression facilitates mitochondrial cristae formation [103]. Similarly, the same group reported that *in vivo* IF1 knockout did not alter the morphology of mitochondrial cristae in various tissues of the IF1 knockout mice under basal condition [81]. It would be intriguing to further examine whether IF1 could help maintain mitochondrial morphology under hypoxic/ischemia conditions because of the acidic conditions required for IF1 activation. Therefore, further investigations that subject animals to IF1 overexpression and knockout under different pathological conditions may help resolve the above inconsistent observations.

The mitochondrial permeability transition, or MPT, refers to the increase in the permeability of the mitochondrial membranes to molecules of less than 1.5 KD, which results from the opening of a mitochondrial permeability transi-
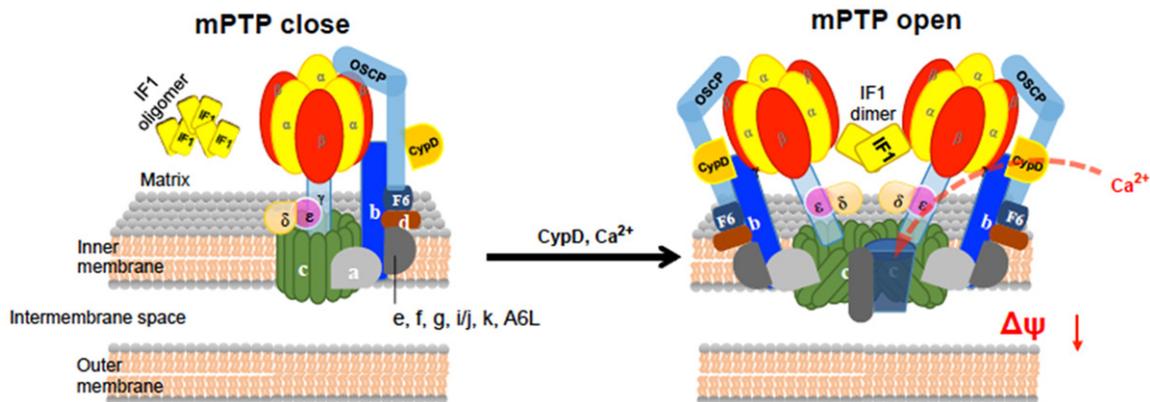
Figure 3. The schematic models of ATP synthase serving as the mitochondrial permeability transition (mPTP). The main mPTP regulator cyclophilin D (CypD) in mitochondrial matrix is a necessary mPTP component responding to stimuli to initiate mPTP opening upon its binding to ATP synthase. The c-ring of the ATP synthase F_o domain acts as the pore of the mPTP. Alternatively, the mitochondrial ATPase inhibitory factor 1 (IF1) dimer binds to the interface between α- and β-subunits of the ATP synthase F_1 domain, inducing the dimerization of the F_1F_o-ATP synthase and forming a pore.

tion pore (mPTP). The mPTP is a protein pore located at the inner membrane of the mitochondria under certain pathological conditions such as myocardial ischemia. The opening of mPTP leads to mitochondrial swelling and cell death of apoptosis or necrosis. Under physiological conditions, oxidative phosphorylation is responsible for ATP production with relatively low ROS production. In contrast, when mPTP is open, the mitochondria not only produces excessive reactive oxygen species (ROS), but also consumes ATP in a futile cycle of efforts to restore the membrane potential, thereby exacerbating cellular damage. However, the exact components of mPTP remain unknown. While cyclophilin D is thought to be an important regulatory protein in determining the opening of mPTP, other earlier identification of mPTP components based mostly on *in vitro* biophysical and biochemical investigations failed to prove their necessity for the proper function of mPTP, at least in various single gene knockout mouse models (see review [104]). In cyclophilin D knockout mice with myocardial ischemia/reperfusion injury, the extracted mitochondria show reduced mPTP opening and therefore show myocardial protective effects [105, 106].

Even though it has long been speculated that ATP synthase may play a key role in mitochondrial permeability transition [107], direct experimental proof has emerged only recently. Based on findings that the c subunit of the ATP synthase is required for MPT-driven mitochondrial

fragmentation and cell death triggered by Ca^{2+} overload and oxidative stress, Bonora *et al* proved that the c subunit of the ATP synthase constitutes a critical component of the mPTP [108]. Another group further provided evidence that cyclosporine A binds to the OSCP and β-subunits of the ATP synthase, indirectly inhibiting the c-subunit (mPTP) pore by inducing a conformational change in ATP synthase that places F_1 over the pore and its conductance [109]. Bcl-xL can be found within the c-subunit of the ATP synthase and is similar to mPTP [91]. The F_1 prevents mPTP opening by being placed over the pore of a leak conductance within the c-subunit ring [91, 109]. This model predicts that cyclophilin D, which is known to bind to OSCP [88], acts on the pore by facilitating the removal of the F_1 from the c-subunit in a CsA-sensitive manner during pore opening [109]. On the other hand, Giorgio *et al* have shown that the binding of cyclophilin D to OSCP facilitates ATP synthase dimerization, which in turn becomes a conductance channel responsible for the opening of mPTP [110]. Apparently, these two models (**Figure 3**) may be interrelated, eg., the formation of ATP synthase dimer may eventually alter the c-subunit conductance. However, further investigations are needed to determine if the c-subunit conductance is dimer dependent. It is also likely that changes in ATP synthase activity are sufficient to alter the formation of ATP synthase related mPTP opening and the detailed correlations should be rigorously investigated.

In general, emerging evidence supports a lasting speculation that ATP synthase acts as the key component of mPTP. However, it is apparent that the molecular and structural details remain scant. Further investigations are required to answer many unresolved questions. For instance, what conformational changes will enable the ATP synthase to act as an mPTP? What is the role of F_1 in regulating the mPTP opening? Does the dimerization of ATP synthase consist of the mPTP or is it merely a required condition for the C-ring channel to work as one? Moreover, *in vivo* evidence is also needed to confirm the *in vitro* findings. Preclinical animal models that illustrate the role of ATP synthase serving as the key components of mPTP will be highly valuable for further development of therapeutic strategies targeting ATP synthase.

Targeting ATP synthase regulation as a therapeutic target for cardiac disorders

Diminished energy supply is a key factor contributing to both the initiation and progressive transition of congestive heart failure (CHF) [2, 111]. The activity of electron transport-chain complexes and ATP synthase capacity are reduced in failing hearts [112-114]. The increased opening of mPTP is one main feature of hearts under ischemia/reperfusion. This impairment in ATP generation and mPTP opening further augments the release of ROS, which exacerbates damages of mitochondria and other important cellular structures. ATP depletion and dysfunctional mitochondria are crucial components of not only impaired contractile function, but also programmed cell death, leading to a remarkable net loss of functional myocardium. Therefore, the mitochondrial function or the energetics of the heart are integrally linked with the causes and phenotype of heart failure. There is a common consensus that improving the myocardial energetic state and preventing excessive mPTP opening should be a therapeutic goal in treating CHF.

Inhibiting the hydrolytic activity of ATP synthase during ischemia without interfering with the synthesis of ATP during normoxic condition is proposed to be therapeutic. Treatment with aurovertin and oligomycin, both inhibit similarly bi-direction of ATP synthase activity showed myocardial protective effects [115]. Similarly, IF-1 is a potential drug target because it is activated under acidic conditions, such as in myocardial ischemia, to enhance ATP reserves in the heart [69, 70]. Preclinical assessments on IF-1 mimetic compounds did show promising results in animal studies [76, 116]. Novel therapies targeting other ATP synthase interacting proteins are also possible to improve myocardial energetics and prevent mPTP opening and mitochondrial dysfunction.

Summary and conclusions

ATP synthase is a fascinating protein complex with an essential role in maintaining life. Its pivotal role is even more obvious in the most energy consuming heart. Several mechanisms are involved in maintaining the steady state activity of ATP synthase, thus determining mitochondrial function via its role in controlling cellular energetics, mitochondrial remodeling and mPTP opening (**Figure 3**). Novel therapies that can correct ATP synthase deficiency, energy depletion and mPTP opening in CHF are highly desired, but these must be first tested in animal models. Given that a majority of the current knowledge about the mitochondrial ATP synthase is based on protein biology, cellular biochemistry and *in vivo* yeast biology, it is highly desirable to gain insights into how this system works in animals and in humans. Studies on preclinical animal models will yield insights into the development of novel therapies targeting the mitochondrial ATP synthase.

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