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Contributed Mini Review

The uniqueness of the plant mitochondrial potassium channel

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The ATP-inhibited Plant Mitochondrial K^+ Channel ($PmitoK_{ATP}$) was discovered about fifteen years ago in Durum Wheat Mitochondria (DWM). $PmitoK_{ATP}$ catalyses the electrophoretic K^+ uniport through the inner mitochondrial membrane; moreover, the co-operation between $PmitoK_{ATP}$ and K^+/H^+ antiporter allows such a great operation of a K^+ cycle to collapse mitochondrial membrane potential ($\Delta\Psi$) and ΔpH, thus impairing protonmotive force (Δp). A possible physiological role of such $\Delta\Psi$ control is the restriction of harmful reactive oxygen species (ROS) production under environmental/oxidative stress conditions. Interestingly, DWM lacking Δp were found to be nevertheless fully coupled and able to regularly accomplish ATP synthesis; this unexpected behaviour makes necessary to recast in some way the classical chemiosmotic model. In the whole, $PmitoK_{ATP}$ may oppose to large scale ROS production by lowering $\Delta\Psi$ under environmental/oxidative stress, but, when stress is moderate, this occurs without impairing ATP synthesis in a crucial moment for cell and mitochondrial bioenergetics. [BMB Reports 2013; 46(8): 391-397]

INTRODUCTION

The existence of an ATP-inhibited mitochondrial potassium channel in plants was firstly shown in Durum Wheat Mitochondria (DWM) (1); it was named Plant $mitoK_{ATP}$ ($PmitoK_{ATP}$) in analogy with the possible animal counterpart [$mitoK_{ATP}$; (2)]. Although the molecular nature of the channel is yet unknown (see 3 for some hypotheses about this point), we have recently confirmed the existence of a DWM cation channel inhibited by ATP referable to the original $PmitoK_{ATP}$ by using patch clamp technique, for the first time successfully applied to plant mitochondria (3). By using swelling technique and/or by following channel-dependent membrane potential changes, other mitochondrial potassium channels were characterized in several plant species such as pea, soybean, three coniferous species, *Arum*, potato, maize and tomato, while less characterized potassium pathways were reported in mitochondria from bread wheat, spelt, rye, barley, spinach, topinambur, triticale, lentil and *Arabidopsis* (see 4-6 and refs therein). These channels may display characteristics different from that of the original $PmitoK_{ATP}$; for example, as reported by Ruy *et al*. (7), in potato, maize and tomato mitochondria, they may show ATP-insensitivity. Moreover, by using electrophysiological measurements in a reconstituted system, three different potassium channels were identified in potato tuber mitochondria: a large conductance Ca^{2+}-activated K^+ channel, the mitoBKCa (8), an ATP-sensitive mitoK, and a large-conductance Ca^{2+}-insensitive and iberiotoxin-sensitive channel (9). So, mitochondrial K^+ channels are widely present in plants and are characterized by different modulation, specificity, conductance and possible physiological role [for recent reviews see Jarmuszkiewicz *et al*. (10) and Pastore *et al*. (11)].

As for DWM, the $PmitoK_{ATP}$ displays a conductance of 150 pS in 150 mM K^+, a strong voltage dependence, relatively low selectivity and ATP inhibition with an IC_{50} of 0.5 mM, as calculated by De Marchi *et al*. (3), or Ki of about 0.3 mM, as obtained by Pastore *et al*. (1). As for modulators, $PmitoK_{ATP}$ activity is mainly increased by superoxide anion, diazoxide and mersalyl, as well as by free fatty acids (FFAs) and their acyl-CoA ester derivatives (12), these latters also modulating the connected activity of the mitochondrial anion channel (13).

THE EFFECT OF $PmitoK_{ATP}$ ON THE MITOCHONDRIAL PROTONMOTIVE FORCE IN DWM

The $PmitoK_{ATP}$ is very active being able to completely collapse electrical membrane potential ($\Delta\Psi$) in DWM isolated *in vitro* in a KCl medium mimicking cell condition; 25 mM KCl are often already sufficient to do this in succinate oxidizing DWM (1, 14). Moreover, the channel may act together with the K^+/H^+ antiporter, very active in plant mitochondria (15), generating a potassium cycle (1) that collapses also ΔpH (6) (Fig. 1).

In this regard, the $PmitoK_{ATP}$ is very different from other mitochondrial potassium channels. In a recent study it was reported that the effect of potato $mitoK_{ATP}$ on mitochondrial $\Delta\Psi$ is very limited (up to few mV) (9) with respect to that of durum wheat channel. In heart mitochondria the increased K^+ influx associated to potassium channel opening was small and it was

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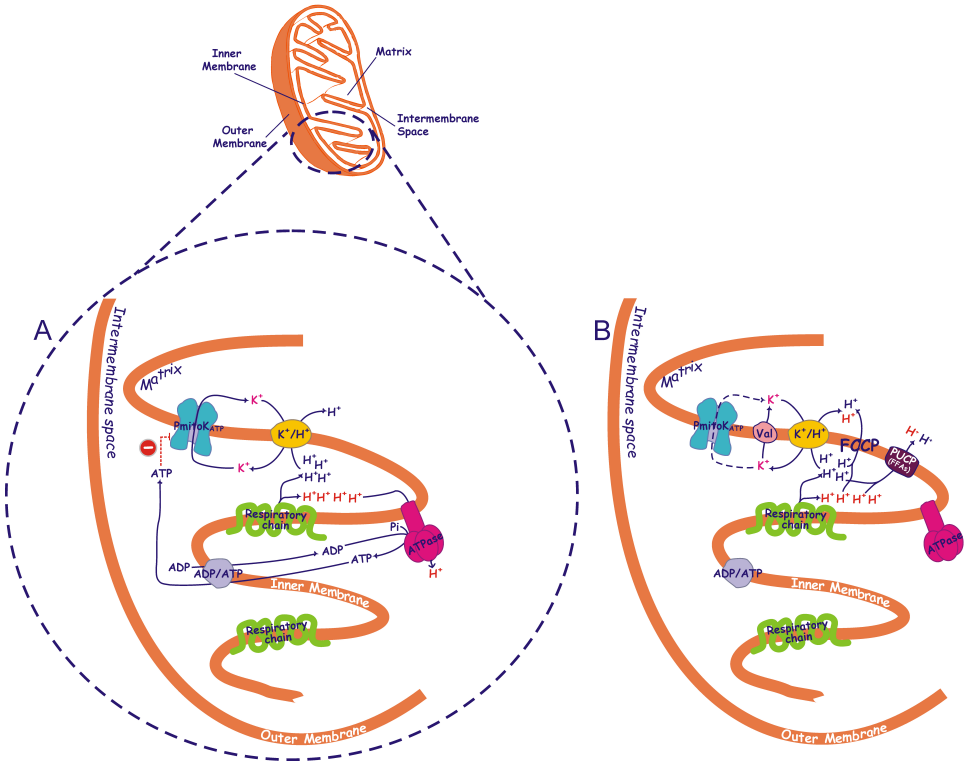

Fig. 1. Possible mechanism of coupling in the absence of measurable protonmotive force mediated by the plant mitochondrial potassium channel. A simplified picture of a durum wheat mitochondrion is reported, with mitochondrial cristae enlarged to schematize the presence in the inner mitochondrial membrane of respiratory chain, ATP synthase (ATPase), ADP/ATP and K^+/H^+ antiporters and ATP-inhibited plant mitochondrial potassium channel (PmitoK$_{ATP}$) (A) as well as of K^+ ionophore valinomycin (Val), plant uncoupling protein (PUCP) activated by free fatty acids (FFAs) and chemical uncoupler carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) (B). Partial inhibition of PmitoK$_{ATP}$ by ATP occurring at the cytosolic face of the channel (1), may control the extent of channel activity. In the presence of high K^+ concentration, the potassium cycle deriving from the cooperation of the PmitoK$_{ATP}$ (A) or valinomycin (B) with K^+/H^+ antiporter may partly or fully uncouple mitochondria, respectively. Protons ejected by the respiratory chain in the course of substrate oxidation are reported in blue and in red; the first ones contribute to the measurable bulk phase $\Delta\Psi$ and ΔpH, while the second ones are assumed to represent a latent non-classically measurable, localized, protonmotive force. The controlled cooperation of PmitoK$_{ATP}$ with K^+/H^+ antiporter may collapse measurable bulk phase $\Delta\Psi$ and ΔpH without excluding the ATP synthase pathway (A). When uncontrolled K^+ uptake by valinomycin bypasses ATP brake, classical uncoupling is observed involving all protons and excluding ATP synthase (B). As expected, uncoupling is also observed when FFAs or FCCP are used (B). The scheme does not consider topology of proteins and interaction sites. For detailed explanation see the text.

able to depolarize by only 1-2 mV (16).

In rat liver mitochondria some $\Delta\Psi$ decrease was observed depending on KCl concentration (up to about 20 mV at 100 mM KCl), but it was compensated by ΔpH increase so that the protonmotive force (Δp) remained almost constant (17); in the same mitochondria Devin *et al*. (18) showed that, for the same respiration rate, Δp was lesser in a KCl medium than in sucrose medium, but the ratio between the amount of phosphorylated ADP and oxygen consumed (ADP/O) did not vary. In respiring yeast mitochondria, addition of KCl in the presence of 4-5 mM phosphate generated some potassium cycle through electrophoretic K^+ entry and electroneutral K^+/H^+ exchange without promoting any uncoupling between respiration and ATP synthesis, but even increasing ATP synthesis on the basis of a compensatory ΔpH increase that drives the activation of phosphate/H^+ cotransporter (19, 20). On the other hand, Castrejón *et al*. (20) and Manon and Guérin (21) showed that at low phosphate concentration (0.4-0.5 mM), mitochondrial uncoupling by KCl occurred with collapse of Δp and dramatic reduction of ADP/O and respiratory control (RC) ratios, as well as of ATP synthesis rate (about -65% than in high phosphate).

THE UNEXPECTED EFFECT OF PmitoK$_{ATP}$ ON THE ATP SYNTHESIS IN DWM

According to Mitchell's chemiosmotic theory the energy-rich intermediate of mitochondrial oxidative phosphorylation (OXPHOS) is the proton gradient across the inner mitochondrial membrane. Its driving force was defined by Mitchell (22,

23) as the protonmotive force (Δp), consisting of an electrical (ΔΨ) and a chemical (ΔpH) part (24). A major prediction of the chemiosmotic model is that the phosphorylation potential and the rate of ATP synthesis should depend on the magnitude of the bulk Δp. As reported above, OXPHOS-dependent ATP synthesis by mitochondria suspended in a KCl medium is actually related to Δp, but, once again, the effect of PmitoK$_{ATP}$ operation is unexpected.

As stated, fully functional DWM that oxidize succinate show negligible bulk phase ΔΨ and ΔpH in high KCl media. Anyway, DWM are equally fully coupled since they preserve ADP/O ratio and are able to regularly accomplish ATP synthesis under conditions that exclude adenylate kinase activity (6). ATP synthesis via OXPHOS has been observed in three different and independent ways, i) by following in continuous ATP synthesis and efflux from mitochondria by using an enzymatic ATP detecting system, ii) by measuring the synthesized ATP at the end of a phosphorylation cycle and iii) by oxygraphic measurements of the RC and ADP/O ratios and of the ATP synthesis rate calculated by multiplying state 3 oxygen uptake rate by ADP/O (6). KCl-treated DWM always showed ATP synthesis statistically equal to control DWM, although showing very low (60-120 mV) ΔΨ and no measurable ΔpH. On the contrary, as expected, classical uncouplers, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP), FFAs (activating the plant uncoupling protein, PUCP) and valinomycin plus KCl are able to completely collapse both Δp and ATP synthesis.

The paradoxical behaviour of DWM may be connected with the ATP and, to a lesser extent, ADP sensitivity of the potassium channel, that we hypothesize may induce a "controlled collapse" of Δp. At this regard, it should be underlined a notable quantitative difference between PmitoK$_{ATP}$ and its mammalian counterpart mitoK$_{ATP}$ regarding ATP inhibition. The mitoK$_{ATP}$ is strongly inhibited by very low ATP concentration ($K_{0.5}$ = 22-40 μM) and by Mg^{2+} in the presence of ATP (25, 26). Since the physiological ATP concentration in mammalian cells is in the millimolar range, this suggests that ATP can hardly modulate the degree of channel opening *in vivo* (26). On the contrary, PmitoK$_{ATP}$ has lower affinity towards ATP with half inhibition of 0.3-0.5 mM (10- to 15-fold lower than in mitoK$_{ATP}$) and is insensitive to Mg^{2+} (1, 3); these properties may allow DWM channel regulation by ATP *in vivo*. Indeed, on the basis of NMR analysis, it was reported that in plant cells the nucleotide triphosphate concentration ranged between 0.9 and 1.2 mM (27) with about 70% of this content represented by ATP (28). Moreover, under severe stress conditions, even a 40% drop of ATP content may be measured (6), thus reaching concentrations able to significantly mitigate channel brake.

The mechanism that induces the "controlled collapse" of Δp is summarized in Fig. 1. It is based on the ability of ATP at physiological concentration to only partially inhibit the channel, being the level of channel opening a function of the changes of cytosolic ATP concentration in the balance with the effect of different activators (see below). Since PmitoK$_{ATP}$ represents the rate-limiting step of the potassium cycle (1), the inhibition by ATP, although partial, lowers the potassium cycle rate, thus preventing complete uncoupling. As a result of the controlled activity of the channel, the potassium cycle may strongly lower measurable bulk phase Δp, but it appears unable to compete with ATP synthase for protons, so the existence of a latent proton movement non-classically detectable may be preserved (Fig. 1A). Consistently, the addition of valinomycin, that overcomes ATP inhibition and maximize potassium uptake and cycle, causes a complete uncoupling. Similarly, classical uncouplers such as FFAs, that activate the PUCP, or FCCP completely uncouple mitochondria (Fig. 1B). In practice, while the classical uncouplers are unable to distinguish among different proton pools, somehow the PmitoK$_{ATP}$/ATP system appears to be able to distinguish the bulk phase Δp from a non-classically detectable driving force for ATP synthesis. The magnitude of this phenomenon will depend on channel opening, *i.e.* on the balance among channel activators and inhibitors. This mechanism is in accordance with that probably operating in other similar biological systems.

Other biological systems troubling classical chemiosmosis

Some other energy-transducing membranes were shown to trouble the statement of classical chemiosmosis. ATP synthesis was detected even in the presence of an inverted ΔpH, alkaline outside, in extreme alkaliphilic bacteria (29). The attenuation of the rate of succinate oxidation resulted in a parallel decrease in the rate of ATP synthesis with little or no change in Δp in bovine hearth submitochondrial particles (30). Light-induced ATP synthesis was found to occur in the absence of an apparent ΔΨ or ΔpH in both *Halobacterium halobium* (31) and thylakoid vesicles (32). As a result of the accumulation of these kind of observations it was speculated that the delocalized transmembrane Δp cannot be the principal driving force for ATP synthesis (33); so, it was hypothesized that a localized rather than delocalized energy transfer between the electron transfer complexes and the ATP synthase may occur (34-37). These findings may be explained by assuming that a proton transfer through direct protein-protein interaction exists (38, 39). Alternatively, protons generated at the surface of the membrane may diffuse laterally through the surface polar groups of phospholipids or the organized water at the surface (40-42). In any case, it has been proposed that the coupling between proton donor and acceptor sites can be direct, without involving the bulk phase, also at a considerable distance of either nanometers or even micrometers (43-46), so, the localized protons could be directly coupled to ATP synthesis when the protons are channeled through the ATP synthase or alternatively exchanged with ions at symports or antiports (46).

THE POSSIBLE PHYSIOLOGICAL ROLE OF PmitoK$_{ATP}$

The control of ΔΨ may allow the control of reactive oxygen species (ROS) production (47), so this property of the

PmitoK$_{ATP}$ sets out its possible physiological role. Really, opening/closure of the channel in a 100 mM KCl medium may vary up to about 35-fold superoxide anion production (6). It is well known that cellular ROS production can be increased as a result of plant exposure to various environmental stresses, thus inducing oxidative stress (48-50). Mitochondria, in particular, were reported to increase ROS generation under drought and salt stress (51). The deriving hypothesis that PmitoK$_{ATP}$ may operate as defense against these stresses in DWM together with alternative oxidase (52) and PUCP (53) was demonstrated: an increase in channel activity up to fourfold in mitochondria purified from osmotic- and salt-stressed durum wheat seedlings and a concurrent decrease (about 60%) of mitochondrial ROS generation was observed (53). Under conditions of hyperosmotic stress an increase of channel activators such as ROS and FFAs/AcylCoA esters (53, 12), the latters deriving from the activation (up to about two times) of a mitochondrial PLA$_2$ (54), is observed. Under moderate hyperosmotic stress conditions inducing a starting cellular oxidative stress, but not a damage on substrate oxidation, on ATP synthesis and mitochondria intactness (55, 56), activation of the channel may induce ΔΨ decrease and control of ROS production, but, according to the mechanism of Fig. 1A, may preserve ATP synthesis just when the cell has much more need of ATP to overcome the insult (Fig. 2A).

One can argue that ATP synthase cannot work under low force condition. Really, ΔΨ and ΔpH are not kinetically equivalent driving forces for ATP synthase. ΔΨ represents the essential driving force for rotation of the "rotor" $\gamma\varepsilon c_n$ of the synthase; one turn of rotation of the $\gamma\varepsilon c_n$ part yields three ATP driven by the translocation of protons through *c* subunits (57 and refs therein). The ΔΨ required is a function of H^+/ATP stoichiometry that depends, in turn, on the number of the c subunits in F_o rotating ring. For example, in *Escherichia coli*, and probably in mammalian mitochondria, 100-120 mV are assumed to be necessary for maximal ATP synthesis by the ATP synthase that has probably 9-10 c subunits, so giving calculated H^+/ATP equal to 3-3.3; anyway, about 70-80 mV are sufficient to obtain midpoint potential (58). Unfortunately, the number of c subunits of ATP synthase in DWM is so far unknown thus preventing H^+/ATP calculation; moreover, possible alternative calculation of thermodynamic H^+/ATP stoichiometry as ΔGp/Δp is unlikely due to an unspecific proton leak of the inner membrane typical of mitochondria, preventing a thermodynamic equilibrium (59). However, it should be noted that in plants, for ATP synthesis by chloroplast ATP synthase, saturation is already obtained at only 50-60 mV, this enzyme having 14 c subunits, so giving calculated H^+/ATP equal to 4.7 (58). This shows that ATP synthases may be able to synthesize ATP at rather low membrane potential. Consistently, *in vivo*, low mitochondrial ΔΨ has been often measured. In plant cells, mitochondrial ΔΨ was estimated on the basis of the sub-

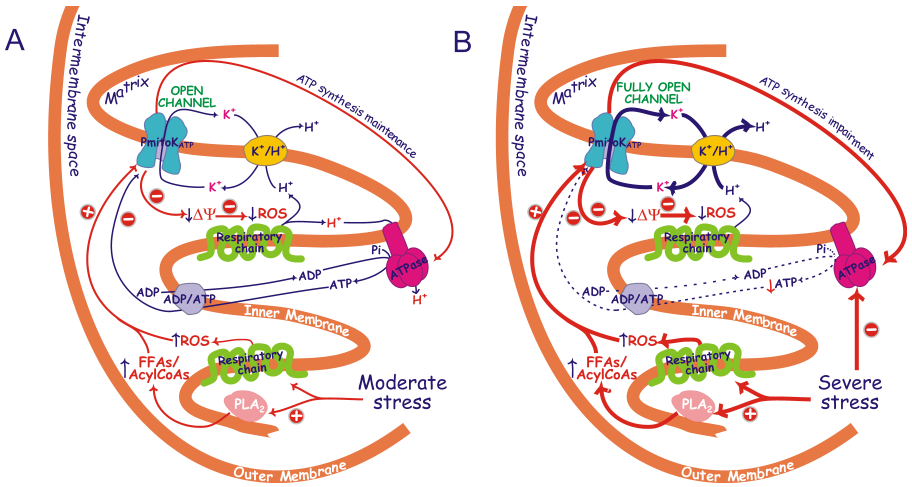

Fig. 2. Possible mechanism of PmitoK$_{ATP}$ modulation by FFAs/acylCoAs, ROS and ATP under moderate (A) and severe (B) hyperosmotic stress conditions. Schematization is as in Fig. 1, PLA$_2$ is a mitochondrial phospholipase A$_2$. Under moderate stress conditions (A) an increase in FFAs due to PLA$_2$ activity and probably of their acylCoA derivatives is observed in DWM, as well as an increased ROS production by respiratory chain, thus leading to PmitoK$_{ATP}$ activation. At the same time, channel inhibition by ATP is able to carefully regulate the rate of K^+ cycle due to the PmitoK$_{ATP}$ - K^+/H^+ combined function, so that the measurable bulk phase ΔΨ/ΔpH (blue protons, see also Fig. 1) can be lowered, thus dampening excess harmful ROS generation; under these conditions, however, ATP synthesis can be maintained using the latent localized protonmotive force (red protons) as proposed in Fig. 1A. Under severe stress (B), ATP synthesis is inhibited; therefore, PmitoK$_{ATP}$ activation by ROS and by PLA$_2$/FFAs/acylCoAs pathway may greatly overcome ATP inhibition. Functioning of fully open PmitoK$_{ATP}$ may actively prevent large scale ROS production, but it may be so high to collapse both bulk phase and localized ΔΨ to such an extent to impair ATP synthesis. The scheme does not consider topology of proteins and interaction sites. The large arrows refer to a more active pathway. Abbreviations are as in Fig. 1. ROS, reactive oxygen species; ↑, increase; ↓, decrease; ⊕, activation; ⊖, inhibition.

cellular ATP/ADP ratio measured by means of rapid subcellular fractionation of barley leaf protoplasts; interestingly, $\Delta\Psi$s ranging from 70 to 95 mV under different physiological conditions were calculated (60). As for mammalian cells, mitochondrial $\Delta\Psi$s of about 105 mV in fibroblasts and 81 mV in neuroblastoma cells were measured (61), these results were obtained by applying a novel method using the combination of conventional fluorescence microscopy and three-dimensional deconvolution by exhaustive photon reassignment. $\Delta\Psi$s ranging from about 100 to 115 mV were measured under different metabolic conditions in perfused rat hearts under high cardiac work (62). Really, *in vitro* values of $\Delta\Psi$ measured in DWM under PmitoK_{ATP} operation in a KCl medium ranged from 60 to 120 mV in different experiments (14, 6, and unpublished data). These $\Delta\Psi$ values fit well with the above measurements *in vivo*, thus suggesting that in DWM under stress, ATP synthesis at suboptimal $\Delta\Psi$ may be possible by an energetic point of view. This may keep in balance mitochondrial/cellular bioenergetics and ROS control under controlled stress conditions as depicted in Fig. 2A.

On the other hand, if the stress becomes so severe as to induce a drop of substrate oxidation (53, 63) and of ATP synthesis (55) inducing remarkable ATP content decrease (6, 56), a substantial decrease of channel inhibition by ATP may be observed. Under these extreme conditions up to about 25 times increase of FFAs has been also observed (12). So, the decrease of the inhibitor along with the notable increase of an activator may strongly activate the channel and the potassium cycle, thus controlling large scale ROS production; anyway, under these conditions the fully open channel may impair ATP synthesis (6) (Fig. 2B).

In conclusion, the uniqueness of the plant PmitoK_{ATP} regarding effects on protonmotive force, ATP synthesis and ROS control may be considered as a complex basic mechanism to adapt mitochondrial and cellular bioenergetics to changing environmental conditions and to oppose oxidative stress.

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