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Review

Mitochondrial consumption of cytosolic ATP: Not so fast

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ABSTRACT

In various pathologic circumstances depolarized mitochondria are thought to precipitate cell death by avidly consuming cytosolic ATP. However, for as long as the inner mitochondrial membrane remains intact the reversal potentials of the adenine nucleotide translocase (ANT) and that of F_0–F_1 ATP synthase are strategically positioned so that they oppose import of cytosolic ATP into the matrix of respiration-impaired mitochondria. This arrangement also seems to protect against a hysteretic consumption of cytosolic ATP accumulating in the mitochondrial matrix, in view of the depolarization caused by inhibition of F_0–F_1 ATP synthase by the endogenous protein IF1, yielding fast ANT reversal rates.

1. Introduction

The F_0–F_1 ATP synthase is a miraculous molecular machine. When embedded in a membrane across which there is sufficient protonmotive force, *pmf*, it generates ATP from ADP and P_i. For a large number of eukaryote species, this comes at a cost of 2.7 protons per ATP molecule [1], though this may not be the case in fungi and possibly other eukaryotes, where the stoichiometry of c subunits in the F_0–F_1 ATP synthase has not been established [2,3]. The energy-requiring step is not the chemical step in ATP synthesis, but as shown by Boyer et al., is the binding of P_i and the release of tightly bound ATP from F_0–F_1 ATP synthase [4]. It seems as though mitochondria are built around this molecular rotary engine to keep it operating in the direction of ATP synthesis by providing the *pmf*. In isolation, i.e. in the absence of a *pmf*, the F_1 portion hydrolyzes ATP avidly [5], hence the name "F_1-ATPase".

The *pmf* consists of a transmembrane potential difference ($\Delta\Psi$m, negative inside), and a transmembrane proton concentration gradient, (ΔpH). In the presence of sufficiently high concentration of inorganic phosphate, as it is the case in vivo [6], ΔpH is very small (<0.15) [7–9], therefore, the directionality of F_0–F_1 ATP synthase is mostly controlled by $\Delta\Psi$m. The value of $\Delta\Psi$m at which F_0–F_1 ATP synthase shifts from ATP-forming to ATP-consuming is termed "reversal potential", E_{rev_ATPase}, and is governed by the concentrations of the participating reactants [10]:

$$E_{rev\_ATPase} = -(316/n) - \left(\frac{2.3RT}{F}/n\right) \cdot \log\left[[MgATP^{2-}]_{in}/[(MgATP]_{in} \cdot [P^-]_{in})\right] - \frac{2.3RT}{F} \cdot pH_{out} - pH_{in} \quad (1)$$

and

$$[P^-]_{in} = [P_{total}]_{in}/(1 + 10^{pH_{in} - pK_{a2}}) \quad (2)$$

where "in" and "out" signify inside and outside the mitochondrial matrix, respectively, "n" is the H^+/ATP coupling ratio [1], R is the universal gas constant 8.31 J mol^{-1} K^{-1}, F is the Faraday constant 9.64×10^4 C mol^{-1}, T is temperature (in Kelvin), $K_{M(ADP)}$ and $K_{M(ATP)}$ are the true affinity constants of Mg^{2+} for ADP and ATP valued $10^{-3.198}$ and $10^{-4.06}$, respectively, according to [11], and [P^-] is the free phosphate concentration (in Molar) given by Eq. (2), where pK_{a2} = 7.2 for phosphoric acid. The F_0–F_1 ATP synthase complex utilizes the Mg^{2+}-bound forms, $MgATP^{2-}$ and MgADP [12]. However, $MgATP^{2-}$ can be expressed as $[ATP^{4-}]free \times [Mg^{2+}]/K_{M(ATP)}$, and MgADP can be expressed as $[ADP^{3-}]free \times [Mg^{2+}]/K_{M(ADP)}$. By incorporating these to Eq. (1), we obtain:

$$E_{rev\_ATPase} = -(316/n) - \left(\frac{2.3RT}{F}/n\right) \cdot \log\left[\left([ATP^{4-}]free_{in} \cdot K_{M(ADP)}\right)/\left([ADP^{3-}]free_{in} \cdot K_{M(ATP)}[P^-]_{in}\right)\right] - \frac{2.3RT}{F} \cdot (pH_{out} - pH_{in})$$

ATP for the hydrolyzing F_1-ATPase may come from matrix substrate-level phosphorylation or cytosolic ATP reserves. Substrate-level phosphorylation in the mitochondrial matrix is

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almost exclusively attributed to succinate-CoA ligase (SUCL or succinate thiokinase or succinyl-CoA synthetase), an enzyme that catalyses the reversible conversion of succinyl-CoA and ADP (or GDP) to CoASH, succinate and ATP (or GTP) [13]. In order for the cytosolic ATP reserves to be tapped, the adenine nucleotide translocase (ANT) must also reverse [10]. The ANT utilizes the free forms of ADP^{3-} and ATP^{4-}, and exhibits its own reversal potential, E_{rev_ANT}, which is in turn governed by the participating reactants [14]:

$$E_{rev\_ANT} = \frac{2.3RT}{F} \cdot \log\left[[ADP^{3-}]free_{out} \cdot [ATP^{4-}]free_{in} / [ADP^{3-}]free_{in} \cdot [ATP^{4-}]free_{out}\right] \quad (4)$$

Eqs. (3) and (4) share the common reactants $[ATP^{4-}]free_{in}$ and $[ADP^{3-}]free_{in}$, therefore, the reversal potentials of the F_0–F_1 ATP synthase and that of ANT can be expressed simultaneously as functions of these common parameters, as shown in Fig. 1 (obtained from reference [10], by permission). From Fig. 1 it is evident that for pathophysiologically relevant matrix ATP/ADP ratio values [14–17] the E_{rev_ATPase} (black triangles) is more negative than E_{rev_ANT} (white triangles), implying that progressively depolarizing mitochondria will first exhibit reversal of the F_0–F_1 ATP synthase, followed by reversal of the ANT. Indeed, computer simulations validated by experimental results in isolated and in situ mitochondria [10] have verified this claim. Mitochondria exhibiting a free matrix ATP/ADP ratio for a given $\Delta\Psi m$ that would place them anywhere within the 'A space' implies that these organelles have their ANT and F_0–F_1 ATP synthase operating in forward mode, i.e. ANT brings ADP into the matrix in exchange for ATP, and F_0–F_1 ATP synthase combines ADP and P_i to generate ATP. Mitochondria exhibiting such values that would place them anywhere within the 'C space' carries the notion that both their ANT and F_0–F_1 ATP synthase operate in reverse mode, i.e. ANT brings ATP into the matrix in exchange for ADP, and F_0–F_1 ATP synthase hydrolyzes ATP to ADP and P_i. Mitochondria exhibiting such values that would place them anywhere within the 'B space' implies that the ANT of these organelles operates in forward mode while the F_0–F_1 ATP synthase operates in reverse mode. I was unable to generate cytosol-mimicking conditions that would place mitochondria within 'D space'; perhaps mitochondria can be depleted of ATP by pyrophosphate, while still retain a considerable membrane potential [18,19]. In my opinion, it is unlikely for mitochondria to exhibit such a high $\Delta\Psi m$ but so low ATP/ADP ratio in vivo, and therefore, this part of the graph exhibits no biological representation.

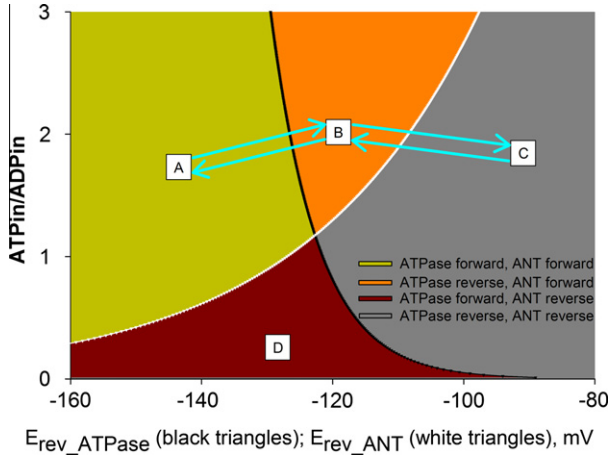

Fig. 1. Computational estimation of E_{rev_ANT} and E_{rev_ATPase} at different free $[ATP]_{in}/[ADP]_{in}$ ratios. (A) ATPase forward, ANT forward; (B) ATP reverse, ANT forward; (C) ATPase reverse, ANT reverse; (D) ATPase forward, ANT reverse. Black triangles represent E_{rev_ATPase}; white triangles represent E_{rev_ANT}. Values were computed for $[ATP]_{out}$ = 1.2 mM, $[ADP]_{out}$ = 10 μM, P_{in} = 0.01 M, n = 3.7 (2.7 plus 1 for the electrogenic ATP^{4-}/ADP^{3-} exchange of the ANT), pH_i = 7.38, and pH_o = 7.25. Traces have been computed by Erev estimator; the software and instructions on how to use it can be downloaded at: http://www.tinyurl.com/Erev-estimator.

From this graph one can deduce the directionalities but not the activities of the ANT and the F_0–F_1 ATP synthase. When $\Delta\Psi m$ approaches the reversal potential of the F_0–F_1 ATP synthase or that of ANT, their respective activities also decrease. However, these activities are neither linearly, nor only related to $\Delta\Psi m$. The ADP–ATP exchange rate of intact mitochondria as a function of $\Delta\Psi m$ and many other parameters have been measured in [9] and [10] and modeled as part of a complete mitochondrial phosphorylation scheme including the ANT, the F_0–F_1 ATP synthase and the P_i carrier, satisfactorily represented by a system of three ordinary differential equations [14]. F_0–F_1 ATP synthase activity has been modeled as a stand-alone reaction in [20]. A typical profile of ADP–ATP exchange rate of intact mitochondria as a function of $\Delta\Psi m$ is shown in Fig. 2. Black squares represent experimental data points obtained from measuring ADP–ATP exchange rates and $\Delta\Psi m$ in parallel in isolated mitochondria titrated with an uncoupler [21]. Similar results have been obtained from in situ mitochondria in permeabilized cells [22]. Such data have been shown to validate our recent model on mitochondrial phosphorylation [14]. For the sake of clarity, the theoretically predicted dependence of F_0–F_1 ATP synthase activity on $\Delta\Psi m$ is omitted from Fig. 2. It cannot be overemphasized that predictions of thermodynamic versus kinetic analyses cannot be used interchangeably; however, the values of $\Delta\Psi m$ at which the F_0–F_1 ATP synthase and ANT yield a net rate of zero deduced by (i) thermodynamic analysis (at $\Delta G = 0$) and (ii) kinetic analysis (elaborated in [14]), are in excellent agreement, [10,14,20,23]. Therefore, when the value of $\Delta\Psi m$ coincides or is very near E_{rev_ANT} or E_{rev_ATPase} deduced from the thermodynamic analysis, it is safe to assume that the activity of the ANT or F_0–F_1 ATP synthase, respectively, is very small.

Mindful of Eqs. (1)–(4) and the computer simulations elaborated in [10] showing that the F_0–F_1 ATP synthase reverses at more negative potentials than the ANT, E_{rev_ATPase} must be somewhere indicated by the red cross indicated in Fig. 2. If the value of $\Delta\Psi m$ becomes less negative in the matrix side than E_{rev_ATPase}, this will cause the F_0–F_1 ATP synthase to reverse. The purpose of this mini-review is to elaborate on the circumstances during which respiration-impaired but still intact mitochondria generate membrane potential by a reverse-operating F_0–F_1 ATP synthase that as explained below, impede further cytosolic ATP import and consumption.

2. Three challenges prior to cytosolic ATP consumption

At this junction, the first challenge emerges: when mitochondria depolarize sufficiently to cause the reversal of F_0–F_1 ATP synthase (i.e. exceeding E_{rev_ATPase}), the membrane potential generated from the protons pumped out of the matrix at the expense of ATP hydrolysis counters further depolarization. However, if the mechanisms causing the initial depolarisation overcome the repolarisation generated by the reversed F_0–F_1 ATP synthase, $\Delta\Psi m$ will reached E_{rev_ANT}.

At this subsequent junction, the second challenge appears: when mitochondria depolarize sufficiently to cause the reversal of the ANT (i.e. exceeding E_{rev_ANT}), membrane potential is generated by exchanging cytosolic ATP^{4-} for matrix ADP^{3-}, antagonizing the depolarization stimulus. Should further depolarization prevail, the third challenge emerges: the more positive $\Delta\Psi m$ becomes, the greater will be the reverse activity of the ANT (indicated by the bidirectional green arrow in Fig. 2). From the model elaborated in reference [14], it is also expected that the less negative $\Delta\Psi m$ becomes, the greater will be the reverse activity of the F_0–F_1 ATP synthase. In reference [10], isolated and in situ mitochondria treated

with inhibitors of the electron transport chain, exhibited magnitudes of $\Delta\Psi m$ that were clamped very near to the predicted E_{rev_ANT} values. Obviously, the reverse operation of the ANT cannot increase $\Delta\Psi m$ higher than E_{rev_ANT}. Accordingly, during these conditions reverse ATP–ADP exchange rates mediated by the ANT were either zero or very small, depending on the type of substrates provided to mitochondria. Substrates can be categorized to those supporting, versus those bypassing substrate-level phosphorylation [10]. Glutamate and α-ketoglutarate are typical respiratory substrates that support ATP formation from substrate-level phosphorylation, while substrates such as succinate and β-hydroxybutyrate bypass this reaction. As shown in Fig. 3A, when mitochondria respired on substrates supporting matrix substrate-level phosphorylation (trace a), there was no cytosolic ATP consumption subsequent to addition of the complex III inhibitor, stigmatellin. When mitochondria respired on succinate, ATP consumption rate ensuing upon addition of stigmatellin was evident, but ~12–15 times slower than ATP production (Fig. 3A, trace b). Still, abolition of ANT activity by carboxyatractyloside caused a prompt and complete depolarization in mitochondria that were provided with succinate (Fig. 3B, trace b). The above results support the conclusion that in the absence of substrate-level phosphorylation, the reverse operation of the ANT was indeed supporting $\Delta\Psi m$ (maintained by the reversals of both the ANT and the F_0–F_1 ATP synthase), but yielding only a very slow rate of ATP entry into the matrix. This is because the generation of membrane potential shifts ATP–ADP exchange rates towards smaller values, as indicated by the direction of the bidirectional green arrow in Fig. 2. In the presence of substrate-level phosphorylation, there was only a very minor depolarization induced by carboxyatractyloside (Fig. 3B, trace a), implying that the ANT was operating near its thermodynamic equilibrium.

From the above considerations we hypothesize that if the reasons for mitochondrial depolarization are not great enough to overcome the membrane potential-generating action of the reverse operation of the ANT and the F_0–F_1 ATP synthase (such as during inhibition of the four complexes of the electron transport chain by drugs or anoxia), mitochondria will consume cytosolic ATP at a very low rate, and only in the absence of matrix substrate-level phosphorylation. This rate will be dictated by the extent of deviation of $\Delta\Psi m$ from E_{rev_ANT} towards more depolarizing values. The greater the deviation, the greater the opposition by the reversed ANT (see green double arrow in Fig. 2). In the presence of sufficient matrix substrate-level phosphorylation, moderately depolarized but intact mitochondria cannot consume cytosolic ATP [10]. Nonetheless, accepting that the mechanism(s) for the initial depolarization can be great enough to overcome the repolarization posed by the reversed F_0–F_1 ATP synthase and the ANT, mitochondria could become significant cytosolic ATP consumers. Such could be the case during activation of a low-conductance permeability or extensive uncoupling [24,25], always assuming that the inner mitochondrial integrity is uncompromised; irrespective of the exogenous factor or endogenous molecular entity mediating a large decrease in $\Delta\Psi m$, the common denominator is the extent of decline in $\Delta\Psi m$ itself, that must overcome the potential-generating action(s) of the ANT and/or the F_0–F_1 ATP synthase. Ironically, IF1, a protein which evolved to limit the extent of ATP consumption by a reversed F_0–F_1 ATP synthase by blocking F_1 [26], leads to robust depolarization [27,28]. This depolarization should allow the ANT to reverse with a high rate and bring cytosolic ATP into the matrix, even though F_0–F_1 ATP synthase is affected by IF1. So how does the purpose of IF1 remain un-defied from a possible hysteretic consumption of cytosolic ATP brought into the matrix by a reversed ANT? As discussed below, inhibition of F_1-ATPase by IF1 is not necessarily complete. But even if the amount of ATP brought into the matrix is only partially consumed by mitochondria due to a strongly reversed ANT, it will remain unavailable for cytosolic ATP-dependent mechanisms. To develop our views on this, we must first elaborate on the regulation of F_0–F_1 ATP synthase activity.

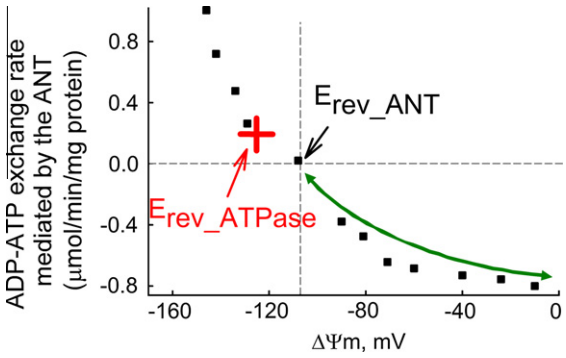

Fig. 2. Plot of the ATP–ADP exchange rate mediated by the ANT versus $\Delta\Psi m$ in isolated rat liver mitochondria depolarized to various voltages by increasing amounts of an uncoupler. Black cross indicates E_{rev_ANT}. Red cross indicates E_{rev_ATPase}. Modified by permission from Elsevier from reference [21].

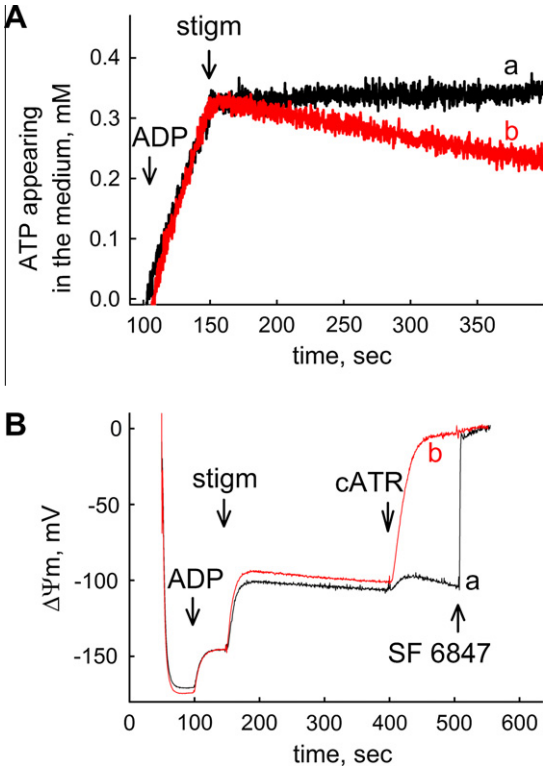

Fig. 3. (A) Reconstructed time courses of ATP appearing in the medium after the addition of 1 mM ADP to rat liver mitochondria respiring on glutamate and malate (a) or succinate plus rotenone (b), followed by addition of the complex III inhibitor, stigmatellin (stigm). (B) Time course of changes in safranine O fluorescence, calibrated to $\Delta\Psi m$ in rat liver mitochondria respiring on glutamate and malate (a) or succinate plus rotenone (b). ADP, stigmatellin and carboxyatractyloside (cATR) were added where indicated. At the end of the experiments, 200 nM of the uncoupler SF 6847 was added to achieve complete depolarization. Modified by permission from the copyrights holder, Federation of American Societies for Experimental Biology, from reference [10].

3. IF1 and hysteretic inhibition of ATP consumption

Regulatory mechanisms of F_0–F_1 ATP synthase activity are divided into those affecting ATP synthesis and those affecting ATP

hydrolysis rates. ATP synthesis activity by the F_0–F_1 ATP synthase can be modulated by changing the magnitude of *pmf*; this can be achieved either by modulation of the complexes of the electron transport chain [29] or the components of the citric acid cycle that provide reducing equivalents to the complexes [30], or by changing the proton permeability of the membrane. Coupling factor B is a protein located in the inner mitochondrial membrane that facilitates ATP synthesis by blocking a proton leak [31]. Recently, IF1 has also been implicated in modulating ATP synthesis activity by a mechanism that involves arrangement of F_0–F_1 ATP synthases in dimer formation, creating more efficient conditions for ATP synthesis by local biophysical factors [32]. Regarding the hydrolytic activity of the F_1-ATPase, four mechanisms are in place aiming at blocking it upon decrease in *pmf*, mediated by: (i) a decrease in matrix ATP concentration, (ii) a decrease in ATP/ADP ratio, (iii) an increase in matrix P_i, and (iv) the endogenous factor IF1 [5,26]. The first two mechanisms converge on the inhibition of the F_1-ATPase by MgADP, reviewed in [5], however, it is not established if this regulation is operational on fully assembled F_0–F_1 ATP synthases within intact mitochondria. The most important regulatory mechanism is sustained by IF1, a protein that is expressed in some tissues of some organisms exhibiting the ability to block the ATP hydrolytic activity of F_1-ATPase [33,34]; however, it must be stressed that IF1 does not operate in an all-or-none manner: the binding and release of this protein in F_1 are governed by both matrix [ATP] and $\Delta\Psi$m, independently from each other [35–37], apart from the effect of matrix pH [38]. Furthermore, inhibition of F_1-ATPase by IF1 may not be complete; matrix ATP inhibits the unbinding of IF1 from F_1-ATPase with a K_i of 0.14 mM [36].

In view of the fact that the action of IF1 on a reverse-operating F_0–F_1 ATP synthase leads to a decrease in $\Delta\Psi$m, the reverse operation of the ANT is augmented. Therefore, ATP is brought into the matrix in exchange for matrix ADP at an accelerated rate, opposing the decrease in matrix [ATP] by the reversed F_1-ATPase, stemming from whatever remaining activity is left due to the incomplete inhibitory action of IF1. This increase in matrix [ATP] will inhibit the unbinding of IF1 from F_1 [36], and also disfavor the reverse mode operation of the ANT [14]. Therefore, cytosolic ATP allocated into the mitochondrial matrix is spared from intramitochondrial hydrolysis, and upon the abrogation of the reasons conferring membrane depolarization on the first place, the only issue remaining is the return of matrix ATP to the cytosol. This is ensured by the following sequence of events: IF1 dissociates from F_1-ATPase upon generation of sufficient proton electrochemical gradient [35–37] and because E_{rev_ANT} is always less negative than E_{rev_ATPase}, during mitochondrial repolarization matrix ATP will first be transported into the cytosol due to ANT reverting towards forward mode of operation, prior to subject to hydrolysis from an F_1-ATPase about to unbind IF1.

4. Do depolarized in situ mitochondria consume cytosolic ATP?

From the above considerations we tender the hypothesis that respiration-impaired depolarized mitochondria cannot deplete cytosolic ATP levels, based on the fact that upon reversal of F_0–F_1 ATP synthase, $\Delta\Psi$m is generated to a level similar to that of E_{rev_ANT}, thereby preventing the ANT from maintaining a forceful import of cytosolic ATP to mitochondria. A suitable way for yielding appreciable ANT reversal rates is to depolarize mitochondria further. Paradoxically, further depolarization can be achieved by blocking the reverse function of the $\Delta\Psi$m-generating F_0–F_1 ATP synthase with the endogenous inhibitor, IF1. But in this case, F_1-ATPase activity is decreased by IF1 and the extent of this block increases with increasing matrix [ATP] and decreasing $\Delta\Psi$m. ATP imported from the cytosol is spared from hydrolysis by the F_1-ATPase and it is ensured return to the cytosol because during repolarization (whenever possible), E_{rev_ANT} is less negative than E_{rev_ATPase}. Therefore, the ANT would expel matrix ATP before the uninhibited F_1-ATPase seizes the opportunity to hydrolyze it.

In line with the above theoretical considerations accompanied with our recent supporting results [10], earlier findings showing that inhibition of the respiratory chain of in situ mitochondria failed to induce a drop in cytosolic [ATP] levels can now be explained [39–42]. Subsequent application of uncouplers led to unquestionable decreases in cytosolic [ATP], as this is expected to induce high reversal rates of the ANT [39]. Limited consumption of cytosolic ATP by in situ mitochondria is also assisted by the fact that when mitochondrial respiration becomes impaired, cells maintain cellular ATP by relying on glycolysis, leading to a build up of lactate that decreases intracellular pH. This drop in pH impairs the activity of the ANT [9] and F_0–F_1 ATP synthase [43] in addition to affecting the concentrations of deprotonated ATP and ADP [14]. All of these effects decrease the ability of mitochondria to consume ATP. Furthermore, in ρ^o cells being unable to carry oxidative phosphorylation [44], the maximum $\Delta\Psi$m value attainable in situ was found to be in the range of −110 to −67 mV [45], in excellent agreement to the predicted E_{rev_ANT} value. In the same cells it was also deduced that the maintenance of $\Delta\Psi$m 'cost' only 13% of the ATP produced by the glycolysis [45]. Yet, for in situ mitochondria of other cell types or even whole organs there is plenty of evidence for cytosolic ATP consumption in various pathologic paradigms [28,46–50]. Apparently, there must be conditions pertaining in situ that counter the principles elaborated above; future efforts should be directed towards their identification.

5. Concluding remarks

Mitochondria have been incriminated in diverse pathologies as ATP consumers. Admittedly, when these circumstances involve strong uncoupling or compromise of the integrity of the inner mitochondrial membrane or other – yet to be identified – conditions all characterized by absence of a *pmf*, F_1-ATPase would do what it does best: hydrolyze ATP. But in respiration-impaired mitochondria with intact inner mitochondrial membranes, if the reverse operation of F_0–F_1 ATP synthase is sustained by ATP provided by matrix substrate-level phosphorylation, $\Delta\Psi$m will be maintained at a value not more negative than E_{rev_ATPase}, while if only cytosolic ATP reserves are being used, the maximum $\Delta\Psi$m value attainable cannot be more negative than that of E_{rev_ANT}, imposing very slow reversal ATP–ADP exchange rates on the translocase. Apparently, the most crucial (though not solitary) determinant of cytosolic ATP consumption by intact mitochondria is the value of $\Delta\Psi$m; like Pythagoras said: "All Is Number".

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