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## ABSTRACT

ATP provided by oxidative phosphorylation supports highly complex and energetically expensive cellular processes. Yet, in several pathological settings, mitochondria could revert to ATP consumption, aggravating an existing cellular pathology. Here we review: i) the pathological conditions leading to ATP hydrolysis by the reverse operation of the mitochondrial  $F_0F_1$ -ATPase, ii) molecular and thermodynamic factors influencing the directionality of the  $F_0F_1$ -ATPase iii) the role of the adenine nucleotide translocase as the intermediary adenine nucleotide flux pathway between the cytosol and the mitochondrial matrix when mitochondria become ATP consumers, iv) the role of the permeability transition pore in bypassing the ANT, thereby allowing the flux of ATP directly to the hydrolyzing  $F_0F_1$ -ATPase v) the impact of the permeability transition pore on glycolytic ATP production, and vi) endo- and exogenous interventions for limiting ATP hydrolysis by the mitochondrial  $F_0F_1$ -ATPase.

## INTRODUCTION

It is now a textbook fact that the most important and fundamental function of mitochondria is i) to provide ATP for cellular functions, ii) to participate in the  $\text{Ca}^{2+}$  homeostasis and iii) to generate and eliminate reactive oxygen species (ROS). In addition, mitochondria could integrate and release lethal signals leading to cell death. ATP provision and  $\text{Ca}^{2+}$  uptake/release are essential physiological functions in brain mitochondria. Compelling evidence have accumulated in the last two decades demonstrating that impaired mitochondrial function is involved in cellular deterioration associated with pathological settings such as ischemia/reperfusion [44], excitotoxicity [110], neurodegenerative diseases or trauma [49]. Since the key features of mitochondria are inherently connected, impaired mitochondrial function usually involves energy deficit, impaired  $\text{Ca}^{2+}$  homeostasis as well as oxidative stress [15]. The latter describes the imbalance of production/elimination of ROS leading to accumulation of these toxic species. In this review we are focusing on the energy deficit related to pathological settings, in which mitochondria not only fail to produce ATP but function as ATP consumers.

In intact mitochondria the electron flow through the respiratory chain generates the proton electrochemical gradient (or protonmotive force, *pmf*; in mV), whose major component is the membrane potential ( $\Delta\Psi_m$ ) being in the range of 150-180 mV, inside negative. *pmf* drives the ATP hydrolyzing  $\text{F}_0\text{F}_1$ -ATPase to function as ATP synthase and promotes the phosphorylation of ADP to ATP. However in mitochondria having an impaired respiration or leaky inner membrane the  $\text{F}_0\text{F}_1$ -ATPase reverses and at the expense of ATP hydrolysis, contributes to the maintenance of  $\Delta\Psi_m$  at a suboptimal level

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by pumping protons out of the matrix. It is obvious that persistent operation of  $F_0F_1$ -ATPase in reverse could consume the cellular ATP reserves driving the cell into an energy crisis eventually leading to cell demise [96]. Likewise, when the condition involving reverse operation of  $F_0F_1$ -ATPase is further complicated by an increased ATP demand, the cellular deterioration is accelerated.

The contribution of mitochondria to cell death has been subject of an intense research for the past two decades. In response to a variety of stressful stimuli, a number of molecules have been identified to emanate from mitochondria, such as cytochrome c, Smac/Diablo, endonuclease G, AIF to name a few, promoting apoptotic cell death [36] [84] [130] [28] [123]. The ability of mitochondria to harbor such an abundance of death-signaling molecules, led them to be likened to “Pandora’s box” [136].

However, although the list of mitochondrial intermembrane proteins triggering apoptosis when released into the cytosol is expanding [128] [123] [68], hard evidence of apoptotic cell death *in vivo* in the adult CNS under pathological conditions such as focal/global ischemia, neurodegeneration or trauma, are lacking [55] [92] [129] [85]. On the other hand, evidence that mitochondrial dysfunction is associated with necrotic cell death is ample [98] [23] [45] [122] [46] [127]. The concept of “energy availability” or even more naively “ATP availability” has been proposed to serve as the switch between apoptosis and necrosis [134] [80]. Based on the property of dysfunctional mitochondria to release death-signaling molecules, as well as to be capable of mounting intense ATP hydrolysis, their pivotal role in dictating the type of cell death was quickly realized [102] [98].

For intact mitochondrial functions the integrity of the mitochondrial membranes is critical; mitochondria having leaky inner membrane reverses the  $F_0F_1$ -ATPase to

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hydrolyze ATP and support  $\Delta\Psi_m$ . For reasons that are incompletely understood, mitochondria possess intrinsic mechanisms for disrupting membrane integrity by recruiting specific proteins to form a pore across the two membranes. This pore, termed “permeability transition pore” (PTP) [60,65,66], is of a sufficient size (cut-off ~1,5 kDa) to allow the uncontrollable passage of solutes and water, that results in swelling and ultimately rupture of the outer membrane since the mitochondrial matrix is hyperosmolar to the cytosol. The identity of the proteins comprising the PTP is debated; the voltage-dependent anion channel (VDAC), hexokinase (HK), creatine kinase (CK), the mitochondrial peripheral benzodiazepine receptor (mPBR), the adenine nucleotide translocase (ANT), the cyclophilin D (cypD) and more recently the phosphate carrier [81], have all been proposed to participate in the formation of the pore [48].

Cyclophilin D is a member of the cyclophilins family [135], that exhibit peptidyl-prolyl *cis/trans* isomerase activity (PPI) and as such it could mediate, in theory, the folding of proteins to the desired conformation in order for the pore to materialize [61].

Unequivocal evidence showing that cyclophilin D plays a major role in the manifestation of necrotic cell death was provided by genetically engineering cyclophilin D knock out mice (cypD KO) [3,8,91,119], which were more resistant to necrotic stimuli than the wild type (WT) littermates. From the studies using cypD KO mice, cyclophilin D emerged as an important drug target, something which is being currently exploited in several clinical trials due to the availability of high-affinity ligands [87]. A fairly obvious reason why maintenance of mitochondrial integrity (through inhibition of the cyclophilin D-dependent PTP) is beneficial to the fate of cell is that mitochondria may not become consumers of cytosolic ATP.

## ATP CONSUMPTION BY MITOCHONDRIA UNDER CONDITONS ASSOCIATED WITH NEURODEGENERATION

The question arises: what are the conditions, which favor the ATP hydrolysis by mitochondria and are relevant to neurodegeneration. The exact molecular mechanism behind neurodegeneration and the sequence of events leading to cell death in the central nervous system are still unknown despite intensive research in the last decades. However compelling evidence support the role of mitochondria in the pathogenesis of a host of neurodegenerative diseases [2] [27] [82] [9]. Oxidative stress and respiratory failure are common features in the pathology of these conditions (reviewed recently by [20]); shifting mitochondria towards consuming rather than synthesizing ATP.

Decreased activity of Complex I, the first complex in the mitochondrial respiratory chain, was found in autopsy samples of the substantia nigra [118] and frontal cortex [105] from patients who suffered from Parkinson's disease (PD). The involvement of complex I deficiency in PD became evident with the discovery that systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces parkinsonian syndromes by selectively inhibiting complex I of the respiratory chain [79] [16] [47]. Parkinsonian phenotypes were also reproduced in laboratory animals with infusion of the well-known complex I inhibitor, rotenone [12]. When the respiratory chain is inhibited,  $\Delta\Psi_m$  would decrease and to prevent this, the  $F_0F_1$ -ATPase starts to function as a proton pump and hydrolyses ATP [121] [96]. Even with nearly complete inhibition of complex I by rotenone,  $\Delta\Psi_m$  is maintained [24] or only partially depolarized [121] in *in situ* mitochondria of isolated nerve terminals (synaptosomes), but collapses in the presence of

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oligomycin, inhibitor of the  $F_0F_1$ -ATPase, clearly demonstrating the role of  $F_0F_1$ -ATPase in the maintenance of  $\Delta\Psi_m$  [121] [24]. Glycolysis is the major source of ATP under this condition and as long as this is sufficient to maintain the ATP-dependent functions, primarily the  $Na^+/K^+$ -ATPase of the plasma membrane, and the ATP hydrolysis by the  $F_0F_1$ -ATPase,  $\Delta\Psi_m$  is not collapsed, the cellular ATP pool is not depleted and cells survive. Glycolytic ATP generation can be accelerated 10 fold in nerve terminals when oxidative phosphorylation is abolished (Kauppinen and Nicholls, 1986) and as a result, ATP/ADP ratio decreases by ~70 % but not collapses (Chinopoulos et al, 1999). Survival of cells with impaired respiratory chain *in vivo* likely depends on the glycolytic capacity; at least that is indicated by studies with cultured cells, where some cell types having high glycolytic capacity can be maintained for hours while others show severe energetic insufficiency in the presence of respiratory inhibitors (reviewed by Budd and Nicholls, 2000).

Complex I was only partially inhibited in PD patients (~30%) but that degree of inhibition was sufficient to decrease the ATP level in nerve terminals [31] [32] suggesting a high flux-control of the respiratory chain by complex I in nerve terminals. It has also been demonstrated that when complex I is inhibited by 25%, the  $F_0F_1$ -ATPase already reverses and supports the maintenance of  $\Delta\Psi_m$  in isolated nerve terminals [22].

Oxidative stress is another factor, which could contribute to the reversal of the  $F_0F_1$ -ATPase under pathological conditions relevant to neurodegeneration. Several lines of evidence support the role of oxidative stress in the pathology of neurodegenerative diseases, which have been reviewed recently by Lin and Beal [82] and Henchcliffe and Beal [63]. Oxidative damage is an early event in the pathology of Alzheimer's disease

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(AD) [100], which precedes amyloid- $\beta$ -peptide deposition in AD animal models [107]. Increased ROS levels were found in AD transgenic mice prior to the appearance of plaques [86]. Signs of oxidative damage were also found in autopsy brain samples from PD patients, such as lipid peroxidation, DNA and RNA damage [33] [137], and oxidative stress has been linked to Lewy body formation [69].

Wide range of effects of oxidative stress on mitochondria have been found in *in vitro* experiments. Relevant to the topic of this review is the report that oxidative stress alone induced by  $H_2O_2$  were without an effect on  $\Delta\Psi_m$  of *in situ* synaptic mitochondria, but greatly reduced  $\Delta\Psi_m$  in the presence of oligomycin clearly showing that under oxidative stress  $\Delta\Psi_m$  is maintained by the reverse operation of the  $F_0F_1$ -ATPase [24]. Various oxidants were also reported to enhance glutamate-induced mitochondrial depolarization [117]. ROS are unlikely to limit directly the mitochondrial respiration as individual components of the respiratory chain are relatively resistant to oxidants [67] [138], however they can limit NADH generation [125] due to inhibition of  $\alpha$ -ketoglutarate dehydrogenase, a key Krebs cycle enzyme [125] [104]. It is of note that the activity of this enzyme was found to be greatly reduced in postmortem brain samples of patients who have suffered from AD [54] [17] or PD [89] [90]. In addition, the enzyme is sensitive to inhibition by  $\beta$ -amyloid [19].

A further important aspect of oxidative stress, which is relevant to neurodegeneration, in particular to PD, is that  $\Delta\Psi_m$  collapses when the oxidative burden is combined with complex I inhibition, while either of these insults themselves influence  $\Delta\Psi_m$  [22]. The underlying mechanism is likely the inhibition of glyceraldehydes-3-phosphate dehydrogenase by ROS [83] [67] resulting in a limited ATP generation in glycolysis.

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Consistent with this,  $\text{H}_2\text{O}_2$  was found to decrease the ATP/ADP ratio in isolated nerve terminals even in the presence of an uncoupler [126], a condition when the accelerated glycolysis provides ATP for the ATP hydrolysis by the reversed  $\text{F}_0\text{F}_1$ -ATPase [121] [1]. As emphasized above and reviewed elsewhere [96] glycolytic ATP generation becomes critical when complex I is inhibited and  $\Delta\Psi_m$  is maintained by the reverse function of the  $\text{F}_0\text{F}_1$ -ATPase.

The repercussions of a bioenergetic insufficiency caused by mitochondrial ATP consumption have been examined in a variety of contexts using brain tissue and was reported to result in an enhanced  $\text{Ca}^{2+}$  entry via the plasma membrane [1] [97], an increased neurotoxic effect of glutamate [99] [64], a progressive deregulation of  $\text{Ca}^{2+}$  and  $\text{Na}^+$  homeostasis [25]. The role of the plasmalemmal  $\text{Na}^+/\text{K}^+$  ATPase, as the major ATP-utilizing reaction in neurons [121], and that of the plasmalemmal and endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase is evident in the loss of the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  homeostasis. Deregulation of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  homeostasis is a key element in initiating/propagating neuronal damage, which is best characterized for glutamate excitotoxicity [35] [30].

It is to note that an increased ATP demand in combination with conditions involving ATP consumption by mitochondria could be fatal for cells. Severe bioenergetic failure developed in glutathione-depleted cerebellar granule neurons when the oxidative burden was combined with an increased cytoplasmic ATP demand imposed either by sustained plasma membrane depolarization or by the excitotoxin kainate [131]. Similarly, oxidative stress combined with a cytoplasmic  $\text{Na}^+$  load resulted in ATP depletion, loss of  $\Delta\Psi_m$  and  $\text{Na}^+$  and  $\text{Ca}^{2+}$  deregulation in nerve terminals [25]. For glutamate excitotoxicity it has been documented by Nicholls and colleagues, using state-of-the-art *in situ* bioenergetic

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approaches that  $\text{Na}^+$  and  $\text{Ca}^{2+}$  load imposes such a large energetic demand on cells that the entire respiratory capacity of *in situ* mitochondria is used [95]. As a result, even a modest restriction in mitochondrial capacity due to a diminished complex I activity, such as that observed in models of Parkinson's disease, or preexisting oxidative stress implicated in a variety of neurodegenerative diseases, greatly potentiate glutamate excitotoxicity, and commit the cell to die [95] [112].

**FACTORS DETERMINING THE ATP CONSUMPTION BY MITOCHONDRIA**

In isolation, the mitochondrial  $\text{F}_0\text{F}_1$ -ATPase vigorously hydrolyzes ATP [50]. This molecular rotor forms ATP only when there is sufficient *pmf* across the membrane in which it is embedded. Ultimately, this depends on the concentration of the reactants participating in the ATP formation/hydrolysis process, namely, *free* matrix [ADP], *free* matrix [Pi], *free* matrix [ATP], matrix pH and intermembrane space pH, as well as the  $\text{H}^+$ /ATP ratio (coupling ratio “n” [124] [29]). The rate as well as the directionality of the  $\text{F}_0\text{F}_1$ -ATPase operation is dictated by the magnitude of the *pmf*. However, the mechanisms by which the  $\text{F}_0\text{F}_1$ -ATPase operate and reverse are not fully understood. It is unclear whether the reverse operation of the ATPase is a mirror image of the forward mode, or it follows a different path [133] [50]. Whatever the exact mechanism of ATP synthesis/hydrolysis may be, a plethora of cytosolic and mitochondrial ATP consuming processes benefit from the high ATP provision by the synthesis operation of the  $\text{F}_0\text{F}_1$ -ATPase, provided that *pmf* is of sufficient magnitude. At 37 °C,  $pmf = \Delta\Psi_m - 60\Delta\text{pH}$ , where  $\Delta\text{pH}$  is the pH gradient across the inner mitochondrial membrane, which could be in the range of 0.8-0 and is dependent on the concentration of matrix Pi [26] [75].  $\Delta\Psi_m$

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and  $\Delta pH$  are far from being static values, even during physiological conditions. At least for  $\Delta\Psi_m$ , it has been shown that mitochondria in a cell exhibit significant fluctuations, some greater than 100 mV [37] [101] [53]. This phenomenon, termed “ $\Delta\Psi_m$  flickering” is observed in mitochondria probed in isolation or *in situ* [101]. Quantitative measurements of  $\Delta\Psi_m$  and/or  $\Delta pH$  during stressful stimuli *in situ* are scarce [94] [13], while some indirect indices of metabolic competence may be misleading [14]; for example, mitochondrial  $Ca^{2+}$  uptake is electrophoretic in nature, however, mitochondria could sequester  $Ca^{2+}$  even when they are depolarized to less than 10% of their full attainable  $\Delta\Psi_m$  [26]. When mitochondria are dysfunctional, respiration is impaired or mitochondria are uncoupled, but the inner membrane permeability remains uncompromised, the extent of *pmf*, hence of  $\Delta\Psi_m$  and  $\Delta pH$ , would dictate whether  $F_0F_1$ -ATPase generates or consumes ATP. The way in which the participating reactants (see above) contribute to this, is yet to be determined. Recently, there is a flare in modeling approaches using the reactants as parameters in an attempt to predict mitochondrial bioenergetics [10] [11] [38] [4] [5]. Using such models, it would be one day possible to predict if  $F_0F_1$ -ATPase operates in the forward or reverse mode during predefined metabolic conditions.

What is the source of ATP for the  $F_0F_1$ -ATPase operating in reverse with uncompromised inner mitochondrial membrane? As discussed above glycolysis is thought to provide ATP for the  $F_0F_1$ -ATPase to hydrolyze and since hydrolysis takes place on the matrix side, both adenine nucleotide translocase (ANT) and  $F_0F_1$ -ATPase must operate in reverse when mitochondria consume cytosolic ATP. However, the operation of ANT and of the  $F_0F_1$ -ATPase are not necessarily in directional synchrony; these molecular entities only

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share matrix [ADP] and [ATP] as common reactants. Cytosolic [ADP] and [ATP] participate in the ANT operation, but not in the reaction catalyzed by the  $F_0F_1$ -ATPase. By the same token, while matrix [Pi], matrix pH and intermembrane space pH, as well as the  $H^+$ /ATP ratio dictate the rate and directionality of the  $F_0F_1$ -ATPase, they are not involved directly in the ANT reaction. Therefore, fluctuation in the concentration of parameters that have no direct influence on the ANT or  $F_0F_1$ -ATPase may result in reversal of the one but not of the other. There are some reports in the literature that suggest a physical link of the  $F_0F_1$ -ATPase to the ANT, that together with the phosphate carrier would form a so-called ATP “synthasome”, (ATPase:Pi transporter:ANT in 1:1:1 ratio) [76] [21] [106]. However, that is at odds with an estimated ratio of ANT/Pi-transporter of ~4; for details on this matter, the reader is referred to a recent review by Martin Klingenberg [74].

From the above considerations, it is apparent that certain values of  $\Delta\Psi_m$  and/or  $\Delta pH$  may allow only the ANT but not the  $F_0F_1$ -ATPase to reverse, and *vice versa*, i.e. the  $F_0F_1$ -ATPase to reverse but not the ANT. Furthermore, the reverse operation of the  $F_0F_1$ -ATPase is subject to regulation, at least in tissues expressing the endogenous IF-1 protein (see below), but for the ANT no ligand-mediated regulation has been described so far. It is therefore fair to assume that there is no directional synchrony in the operation of ANT and  $F_0F_1$ -ATPase. Therefore it is intriguing to consider that ATP generated by substrate-level phosphorylation within the matrix might supply ATP to the  $F_0F_1$ -ATPase. Two reactions are capable of substrate-level phosphorylation; the mitochondrial phosphoenolpyruvate carboxykinase (PEPCK), and the succinate-CoA ligase (SUCL or succinate thiokinase or succinyl-CoA synthetase). Mitochondrial PEPCK, by its reverse

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reaction forming phosphoenolpyruvate and using GTP has been proposed to participate in the transfer of the phosphorylation potential from mitochondria to cytosol [78]. SUCL catalyses the reversible conversion of succinyl-CoA and ADP or GDP to CoASH, succinate and ATP or GTP [71]. The enzyme is a heterodimer, being composed of an invariant  $\alpha$  subunit encoded by the gene *SUCLG1*, and a substrate-specific  $\beta$  subunit, encoded by either *SUCLA2* or *SUCLG2*. This dimer combination results in either an ADP-forming SUCL (A-SUCL, EC 6.2.1.5) or a GDP-forming SUCL (G-SUCL, EC 6.2.1.4). The  $\beta$  subunit thus determines the substrate specificity of the enzyme. Both  $\beta$  subunits are widely expressed, with *SUCLG2* predominantly expressed in anabolic tissues such as liver, and *SUCLA2* in catabolic tissues such as brain and skeletal muscle [71] [72] [78]. *SUCLG1* is ubiquitously expressed [78]. Therefore, it is possible that the availability of succinyl-CoA for the succinyl-CoA ligase in ailing brain mitochondria would determine whether matrix substrate-level phosphorylation could provide ATP for  $F_0F_1$ -ATPase working in reverse.

**THE ROLE OF ANT AS THE INTERMEDIARY ADENINE NUCLEOTIDE FLUX PATHWAY BETWEEN THE CYTOSOL AND THE MITOCHONDRIAL MATRIX**

Theoretically, the rate of ATP provision to the  $F_0F_1$ -ATPase would be the sum of the rate of matrix substrate-level phosphorylation, and that by which ATP is supplied by the operation of the ANT. The rate and directionality of the reactions in the citric acid cycle would determine the rate of production of ATP by SUCL. Likewise, the rate of ATP influx into the matrix depends both on the rate of production in glycolysis as well as on

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the rate of transport by the ANT. The contribution of citric acid cycle to ATP production by SUCL and the regulation of the rate of ATP production by glycolysis is beyond the scope of this review. Assuming that glycolytic ATP production is unhindered and upregulated due to Pasteur effect, ANT becomes the intermediary entity between cytosolic ATP provision and the consuming  $F_0F_1$ -ATPase. The maximum turnover number (defined as the maximum number of substrate molecules that an enzyme can convert to product per catalytic site per unit of time) of  $F_0F_1$ -ATPase is approximately  $50\text{-}100\text{ s}^{-1}$  [108] [50]. The maximum turnover number of ANT in the forward mode is in the range of  $6\text{-}99\text{ s}^{-1}$ , depending on isoform distribution, among other conditions [26]. Regarding the reverse mode of the ANT, there are few reports in the literature [74], estimating turnover numbers in a relevant range, though mathematical modeling of the ANT in isolation predicts almost double rates [88]. Assuming that the rate of ATP influx by the ANT may be equal or slower than the rate of ATP hydrolysis by the  $F_0F_1$ -ATPase, the ANT may become limiting if the amount of ANTs present in mitochondria is equal or lower than that of the  $F_0F_1$ -ATPase. The possible limitation by ANT imposed on the consuming  $F_0F_1$ -ATPase is not to be confused with the higher “flux control coefficient” of the ANT than that of the  $F_0F_1$ -ATPase; the flux control coefficient is defined (for infinitesimally small changes) as the percentage change in the steady state rate of the pathway divided by the percentage change in the enzyme level causing the flux change. ANT exhibits a large flux control coefficient [73], however, this has been defined for the rate of oxygen consumption, not for ATP formation or hydrolysis by the  $F_0F_1$ -ATPase. Considering the above, we propose that in the absence of matrix substrate-level phosphorylation, ANT gains a critical role in conveying ATP into the matrix at a

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sufficient rate for the  $F_0F_1$ -ATPase to hydrolyze. Since pertaining metabolic conditions as well as ANT isoform distribution play a major role in the turnover number of ANT [26], it follows that the very same conditions are able to affect the rate of ATP hydrolysis by the  $F_0F_1$ -ATPase.

**THE ROLE OF PTP IN MITOCHONDRIAL ATP CONSUMPTION**

So far, we have considered factors, which are important for ATP consumption by mitochondria having impaired respiration but uncompromised inner membrane permeability. Next, we address the role of PTP as i) a bypass of ANT, allowing the delivery of cytosolic ATP directly to the consuming  $F_0F_1$ -ATPase, and ii) an impediment to glycolytic ATP production.

As mentioned above, PTP is of a sufficient size (cut-off ~1,5 kDa) to allow the uncontrollable passage of solutes and water across the inner mitochondrial membrane, resulting in cell death as observed in neurodegeneration [49]. Equally fit, interactions of Bax and tBid with VDAC [7] [6] or Bax alone [120] also lead to formation of pores with mega-conductances. Pore opening in mitochondria would obviously lead to a collapse of  $\Delta\Psi_m$  and  $\Delta pH$ , and to full reversal of the  $F_0F_1$ -ATPase, with a direct access to cytosolic ATP. This has the following two ramifications: i) ANT no longer controls ATP hydrolysis by the  $F_0F_1$ -ATPase, and ii) ADP and  $P_i$ , the hydrolysis products of the  $F_0F_1$ -ATPase, are diluted in the cytosol-matrix continuum; therefore, the impediment of the reversal of  $F_0F_1$ -ATPase, which is otherwise exerted by ADP and  $P_i$ , is lost [57]. A secondary consequence of chaotropic ADP and  $P_i$  production in the cytosol-matrix continuum, may be that ADP is no longer available at sites where it could stimulate

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glycolysis. Although at present this statement is purely speculative, the apposition of mitochondria to other organelles for the sake of optimal operation is a well-established phenomenon [37] [111]. ADP is known to accumulate in the intermembrane space resulting in steep gradients across the outer mitochondrial membrane, playing a significant role in efficient oxidative phosphorylation [52] [51]. In addition, the “wiring” of mitochondrial ATP production to nuclear utilization has been also described [40] [39], involving the concerted action of adenylate kinase, nucleoside diphosphate kinase and creatine kinase. Finally, matrix adenine nucleotide microcompartmentation has been proposed [132] [58] [103], though never unequivocally proven [62] [59]. It is therefore highly probable, that compartmentalized provision of ADP to the cytosol may assist in the stimulation of glycolysis partially compensating for the impaired mitochondrial ATP generation in ATP-consuming mitochondria. Besides the above speculation, there are two phenomena that are highly likely to counteract the stimulation of glycolysis, when mitochondria experience PTP: i) the spill of citrate from mitochondria to the cytosol, which is well-known to play a major role in the regulation of glycolysis by inhibiting phosphofructkinase-1 and ii) the depletion of  $\text{NAD}^+$  [34], which is otherwise required for the glyceraldehyde-3-phosphate dehydrogenase reaction. Due to opening of PTP,  $\text{NAD}^+$  leaks from the matrix to the cytosol, where a mitochondria-associated  $\text{NAD}^+$ -glycohydrolase degrades it to as low as 92% of the original values [34]. The remaining 8% [ $\text{NAD}^+$ ] is likely to be insufficient for glyceraldehyde-3-phosphate dehydrogenase reaction. The additional contribution of the catabolic products of this  $\text{NAD}^+$ -glycohydrolase to cell demise has been reviewed elsewhere [23]. It is to note here that loss of pyridine nucleotides from mitochondria is also crucial in our recent finding

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showing that in isolated mitochondria exhibiting PTP, the production of ROS is not increased, as expected, but rather decreased by  $\text{Ca}^{2+}$  [77]. Finally, it has also been proposed that a diminished provision of ATP for hexokinase may limit phosphorylation of glucose upon entry to the cytosol, thereby impeding glycolytic flux at its most proximal step [41]. Therefore, in the presence of PTP a diminished glucose phosphorylation, a decreased glyceraldehyde-3-phosphate dehydrogenase flux and an impaired PFK-1 activation could collectively lead to a spiral towards decreased ATP production by the glycolysis.

**INTERVENTIONS FOR LIMITING MITOCHONDRIAL ATP HYDROLYSIS**

With the exception of a recently discovered possible phosphorylation site [42], ANT does not appear to be subject to ligand- or allosteric- type of regulation. However, nature has developed at least one mechanism for preventing over-consumption of ATP by the  $\text{F}_0\text{F}_1$ -ATPase, during extensive mitochondrial depolarization, substantiated by the IF-1 protein [109] [18]. IF-1 was discovered in 1963 by Pullman and Monroy as a soluble, heat-stable protein present in bovine heart mitochondria, that inhibited the ATP hydrolase activity of the  $\text{F}_0\text{F}_1$ -ATPase [109]. Several additional mechanisms of regulation of ATPase are known to exist [93] [43] [57], however, they are not as well characterized as IF-1 [57]. IF-1 exhibits strong tissue and species distribution, and also variable level of expression and affinity for the  $\text{F}_0\text{F}_1$ -ATPase [116] [115] [114]. The contribution of IF-1 in neurodegeneration has not been addressed, nonetheless, the possible benefit from the existence of this molecule is reflected in the fact that during myocardial ischemia, 90% of the total ATP content could be inefficiently hydrolyzed, which can be diminished to less

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than 50% due to inhibition of the reverse operation of  $F_0F_1$ -ATPase by IF-1 [56] [70] [113]. Furthermore, it was recently demonstrated that overexpression of IF-1 is protective against ischemic injury [18]. What is rather surprising, is that the inhibition of  $F_0F_1$ -ATPase by IF-1 is incomplete [56], thereby mitochondrial and cytosolic ATP pools are not completely rescued. Mindful of this oddity, Grover and colleagues have generated a synthetic  $F_0F_1$ -ATPase inhibitor, called BMS-199264, that affects only the reverse operation [56] [57]. Accordingly, BMS-199264 was shown to be strongly cardioprotective in ischemia/reperfusion paradigms, without affecting ATP synthesis [57].

## CONCLUSIONS

The multitude of cytosolic as well as matrix ATP consuming processes, all dependent on the provision of ATP by oxidative phosphorylation attest to the complexity of eukaryotic life. However, besides some important differences regarding the integrity of the inner mitochondrial membrane, it remained an inherent ability of mitochondria to consume cytosolic and/or mitochondrial ATP during cell stress. This is probably one of the best examples of familiar knowledge: what can make you, can also break you.

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## Reference List

1. K.E. Akerman and D.G. Nicholls, ATP depletion increases Ca<sup>2+</sup> uptake by synaptosomes, *FEBS Lett.* 135 (1981) pp. 212-214.
2. J.K. Andersen, Oxidative stress in neurodegeneration: cause or consequence?, *Nat. Med.* 10 Suppl (2004) p. S18-S25.
3. C.P. Baines, R.A. Kaiser, N.H. Purcell, N.S. Blair, H. Osinska, M.A. Hambleton, E.W. Brunskill, M.R. Sayen, R.A. Gottlieb, G.W. Dorn, J. Robbins, and J.D. Molkenin, Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death, *Nature* 434 (2005) pp. 658-662.
4. R.S. Balaban, Maintenance of the metabolic homeostasis of the heart: developing a systems analysis approach, *Ann. N. Y. Acad. Sci.* 1080 (2006) pp. 140-153.
5. R.S. Balaban, Modeling mitochondrial function, *Am. J. Physiol Cell Physiol* 291 (2006) p. C1107-C1113.
6. J. Banerjee and S. Ghosh, Bax increases the pore size of rat brain mitochondrial voltage-dependent anion channel in the presence of tBid, *Biochem. Biophys. Res. Commun.* 323 (2004) pp. 310-314.
7. J. Banerjee and S. Ghosh, Phosphorylation of rat brain mitochondrial voltage-dependent anion as a potential tool to control leakage of cytochrome c, *J. Neurochem.* 98 (2006) pp. 670-676.
8. E. Basso, L. Fante, J. Fowlkes, V. Petronilli, M.A. Forte, and P. Bernardi, Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D, *J. Biol. Chem.* 280 (2005) pp. 18558-18561.
9. M.F. Beal, Bioenergetic approaches for neuroprotection in Parkinson's disease, *Ann. Neurol.* 53 Suppl 3 (2003) p. S39-S47.
10. D.A. Beard, A biophysical model of the mitochondrial respiratory system and oxidative phosphorylation, *PLoS. Comput. Biol.* 1 (2005) p. e36.

11. D.A. Beard and H. Qian, Thermodynamic-based computational profiling of cellular regulatory control in hepatocyte metabolism, *Am. J. Physiol Endocrinol. Metab* 288 (2005) p. E633-E644.
12. R. Betarbet, T.B. Sherer, G. MacKenzie, M. Garcia-Osuna, A.V. Panov, and J.T. Greenamyre, Chronic systemic pesticide exposure reproduces features of Parkinson's disease, *Nat. Neurosci.* 3 (2000) pp. 1301-1306.
13. A.P. Bolshakov, M.M. Mikhailova, G. Szabadkai, V.G. Pinelis, N. Brustovetsky, R. Rizzuto, and B.I. Khodorov, Measurements of mitochondrial pH in cultured cortical neurons clarify contribution of mitochondrial pore to the mechanism of glutamate-induced delayed Ca<sup>2+</sup> deregulation, *Cell Calcium* 43 (2008) pp. 602-614.
14. J.B. Brocard, M. Tassetto, and I.J. Reynolds, Quantitative evaluation of mitochondrial calcium content in rat cortical neurones following a glutamate stimulus, *J. Physiol* 531 (2001) pp. 793-805.
15. P.S. Brookes, Y. Yoon, J.L. Robotham, M.W. Anders, and S.S. Sheu, Calcium, ATP, and ROS: a mitochondrial love-hate triangle, *Am. J. Physiol Cell Physiol* 287 (2004) p. C817-C833.
16. R.S. Burns, P.A. LeWitt, M.H. Ebert, H. Pakkenberg, and I.J. Kopin, The clinical syndrome of striatal dopamine deficiency. Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), *N. Engl. J. Med.* 312 (1985) pp. 1418-1421.
17. R.F. Butterworth and A.M. Besnard, Thiamine-dependent enzyme changes in temporal cortex of patients with Alzheimer's disease, *Metab Brain Dis.* 5 (1990) pp. 179-184.
18. M. Campanella, E. Casswell, S. Chong, Z. Farah, M.R. Wieckowski, A.Y. Abramov, A. Tinker, and M.R. Duchen, Regulation of mitochondrial structure and function by the F<sub>1</sub>F<sub>0</sub>-ATPase inhibitor protein, IF1, *Cell Metab* 8 (2008) pp. 13-25.
19. C.S. Casley, L. Canevari, J.M. Land, J.B. Clark, and M.A. Sharpe, Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities, *J. Neurochem.* 80 (2002) pp. 91-100.
20. R.K. Chaturvedi and M.F. Beal, Mitochondrial approaches for neuroprotection, *Ann. N. Y. Acad. Sci.* 1147 (2008) pp. 395-412.
21. C. Chen, Y. Ko, M. Delannoy, S.J. Ludtke, W. Chiu, and P.L. Pedersen, Mitochondrial ATP synthasome: three-dimensional structure by electron microscopy of the ATP synthase in complex formation with carriers for Pi and ADP/ATP, *J. Biol. Chem.* 279 (2004) pp. 31761-31768.

22. C. Chinopoulos and V. Adam-Vizi, Mitochondria deficient in complex I activity are depolarized by hydrogen peroxide in nerve terminals: relevance to Parkinson's disease, *J. Neurochem.* 76 (2001) pp. 302-306.
23. C. Chinopoulos and V. Adam-Vizi, Calcium, mitochondria and oxidative stress in neuronal pathology. Novel aspects of an enduring theme, *FEBS J.* 273 (2006) pp. 433-450.
24. C. Chinopoulos, L. Tretter, and V. Adam-Vizi, Depolarization of in situ mitochondria due to hydrogen peroxide-induced oxidative stress in nerve terminals: inhibition of alpha-ketoglutarate dehydrogenase, *J. Neurochem.* 73 (1999) pp. 220-228.
25. C. Chinopoulos, L. Tretter, A. Rozsa, and V. Adam-Vizi, Exacerbated responses to oxidative stress by an Na(+) load in isolated nerve terminals: the role of ATP depletion and rise of [Ca(2+)](i), *J. Neurosci.* 20 (2000) pp. 2094-2103.
26. C. Chinopoulos, S. Vajda, L. Csanady, M. Mandi, K. Mathe, and V. Adam-Vizi, A novel kinetic assay of mitochondrial ATP-ADP exchange rate mediated by the ANT, *Biophys. J.* 96 (2009) pp. 2490-2504.
27. S.J. Chinta and J.K. Andersen, Redox imbalance in Parkinson's disease, *Biochim. Biophys. Acta* 1780 (2008) pp. 1362-1367.
28. J. Cote, J. Renaud, and A. Ruiz-Carrillo, Recognition of (dG)n.(dC)n sequences by endonuclease G. Characterization of the calf thymus nuclease, *J. Biol. Chem.* 264 (1989) pp. 3301-3310.
29. R.L. Cross and V. Muller, The evolution of A-, F-, and V-type ATP synthases and ATPases: reversals in function and changes in the H+/ATP coupling ratio, *FEBS Lett.* 576 (2004) pp. 1-4.
30. A. Czyz, G. Baranauskas, and L. Kiedrowski, Instrumental role of Na+ in NMDA excitotoxicity in glucose-deprived and depolarized cerebellar granule cells, *J. Neurochem.* 81 (2002) pp. 379-389.
31. G.P. Davey, L. Canevari, and J.B. Clark, Threshold effects in synaptosomal and nonsynaptic mitochondria from hippocampal CA1 and paramedian neocortex brain regions, *J. Neurochem.* 69 (1997) pp. 2564-2570.
32. G.P. Davey, S. Peuchen, and J.B. Clark, Energy thresholds in brain mitochondria. Potential involvement in neurodegeneration, *J. Biol. Chem.* 273 (1998) pp. 12753-12757.
33. D.T. Dexter, C.J. Carter, F.R. Wells, F. Javoy-Agid, Y. Agid, A. Lees, P. Jenner, and C.D. Marsden, Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease, *J. Neurochem.* 52 (1989) pp. 381-389.

34. F. Di Lisa, R. Menabo, M. Canton, M. Barile, and P. Bernardi, Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD<sup>+</sup> and is a causative event in the death of myocytes in postischemic reperfusion of the heart, *J. Biol. Chem.* 276 (2001) pp. 2571-2575.
35. R. Dingledine, K. Borges, D. Bowie, and S.F. Traynelis, The glutamate receptor ion channels, *Pharmacol. Rev.* 51 (1999) pp. 7-61.
36. C. Du, M. Fang, Y. Li, L. Li, and X. Wang, Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition, *Cell* 102 (2000) pp. 33-42.
37. M.R. Duchen, A. Leysens, and M. Crompton, Transient mitochondrial depolarizations reflect focal sarcoplasmic reticular calcium release in single rat cardiomyocytes, *J. Cell Biol.* 142 (1998) pp. 975-988.
38. J. Dzbek and B. Korzeniewski, Control over the contribution of the mitochondrial membrane potential ( $\Delta\Psi$ ) and proton gradient ( $\Delta\text{pH}$ ) to the protonmotive force ( $\Delta\mu$ ). In silico studies, *J. Biol. Chem.* 283 (2008) pp. 33232-33239.
39. P.P. Dzeja, R. Bortolon, C. Perez-Terzic, E.L. Holmuhamedov, and A. Terzic, Energetic communication between mitochondria and nucleus directed by catalyzed phosphotransfer, *Proc. Natl. Acad. Sci. U. S. A* 99 (2002) pp. 10156-10161.
40. P.P. Dzeja and A. Terzic, Phosphotransfer networks and cellular energetics, *J. Exp. Biol.* 206 (2003) pp. 2039-2047.
41. M. Erecinska, D. Nelson, J. Deas, and I.A. Silver, Limitation of glycolysis by hexokinase in rat brain synaptosomes during intense ion pumping, *Brain Res.* 726 (1996) pp. 153-159.
42. J. Feng, M. Zhu, M.C. Schaub, P. Gehrig, B. Roschitzki, E. Lucchinetti, and M. Zaugg, Phosphoproteome analysis of isoflurane-protected heart mitochondria: phosphorylation of adenine nucleotide translocator-1 on Tyr194 regulates mitochondrial function, *Cardiovasc. Res.* 80 (2008) pp. 20-29.
43. B.A. Feniouk and M. Yoshida, Regulatory mechanisms of proton-translocating F(O)F (1)-ATP synthase, *Results Probl. Cell Differ.* 45 (2008) pp. 279-308.
44. G. Fiskum, A.N. Murphy, and M.F. Beal, Mitochondria in neurodegeneration: acute ischemia and chronic neurodegenerative diseases, *J. Cereb. Blood Flow Metab* 19 (1999) pp. 351-369.

45. G. Fiskum, R.E. Rosenthal, V. Vereczki, E. Martin, G.E. Hoffman, C. Chinopoulos, and A. Kowaltowski, Protection against ischemic brain injury by inhibition of mitochondrial oxidative stress, *J. Bioenerg. Biomembr.* 36 (2004) pp. 347-352.
46. G. Fiskum, A. Starkov, B.M. Polster, and C. Chinopoulos, Mitochondrial mechanisms of neural cell death and neuroprotective interventions in Parkinson's disease, *Ann. N. Y. Acad. Sci.* 991 (2003) pp. 111-119.
47. F. Fornai, O.M. Schluter, P. Lenzi, M. Gesi, R. Ruffoli, M. Ferrucci, G. Lazzeri, C.L. Busceti, F. Pontarelli, G. Battaglia, A. Pellegrini, F. Nicoletti, S. Ruggieri, A. Paparelli, and T.C. Sudhof, Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) pp. 3413-3418.
48. M. Forte and P. Bernardi, Genetic dissection of the permeability transition pore, *J. Bioenerg. Biomembr.* 37 (2005) pp. 121-128.
49. H. Friberg and T. Wieloch, Mitochondrial permeability transition in acute neurodegeneration, *Biochimie* 84 (2002) pp. 241-250.
50. Y.Q. Gao, W. Yang, and M. Karplus, A structure-based model for the synthesis and hydrolysis of ATP by F1-ATPase, *Cell* 123 (2005) pp. 195-205.
51. F.N. Gellerich, The role of adenylate kinase in dynamic compartmentation of adenine nucleotides in the mitochondrial intermembrane space, *FEBS Lett.* 297 (1992) pp. 55-58.
52. F.N. Gellerich, M. Kapischke, W. Kunz, W. Neumann, A. Kuznetsov, D. Brdiczka, and K. Nicolay, The influence of the cytosolic oncotic pressure on the permeability of the mitochondrial outer membrane for ADP: implications for the kinetic properties of mitochondrial creatine kinase and for ADP channelling into the intermembrane space, *Mol. Cell Biochem.* 133-134 (1994) pp. 85-104.
53. A.A. Gerencser and V. Adam-Vizi, Mitochondrial Ca<sup>2+</sup> dynamics reveals limited intramitochondrial Ca<sup>2+</sup> diffusion, *Biophys. J.* 88 (2005) pp. 698-714.
54. G.E. Gibson, K.F. Sheu, J.P. Blass, A. Baker, K.C. Carlson, B. Harding, and P. Perrino, Reduced activities of thiamine-dependent enzymes in the brains and peripheral tissues of patients with Alzheimer's disease, *Arch. Neurol.* 45 (1988) pp. 836-840.
55. P. Golstein and G. Kroemer, Cell death by necrosis: towards a molecular definition, *Trends Biochem. Sci.* 32 (2007) pp. 37-43.

56. G.J. Grover and J. Malm, Pharmacological profile of the selective mitochondrial F1F0 ATP hydrolase inhibitor BMS-199264 in myocardial ischemia, *Cardiovasc. Ther.* 26 (2008) pp. 287-296.
57. G.J. Grover, P.A. Marone, L. Koetzner, and D. Seto-Young, Energetic signalling in the control of mitochondrial F1F0 ATP synthase activity in health and disease, *Int. J. Biochem. Cell Biol.* 40 (2008) pp. 2698-2701.
58. H.C. Hamman and R.C. Haynes, Jr., Elevated intramitochondrial adenine nucleotides and mitochondrial function, *Arch. Biochem. Biophys.* 223 (1983) pp. 85-94.
59. K.J. Hartung, G. Bohme, and W. Kunz, Involvement of intramitochondrial adenine nucleotides and inorganic phosphate in oxidative phosphorylation of extramitochondrially added adenosine-5'-diphosphate, *Biomed. Biochim. Acta* 42 (1983) pp. 15-26.
60. R.A. Haworth and D.R. Hunter, The Ca<sup>2+</sup>-induced membrane transition in mitochondria. II. Nature of the Ca<sup>2+</sup> trigger site, *Arch. Biochem. Biophys.* 195 (1979) pp. 460-467.
61. L. He and J.J. Lemasters, Regulated and unregulated mitochondrial permeability transition pores: a new paradigm of pore structure and function?, *FEBS Lett.* 512 (2002) pp. 1-7.
62. H.W. Heldt and E. Pfaff, Adenine nucleotide translocation in mitochondria. Quantitative evaluation of the correlation between the phosphorylation of endogenous and exogenous ADP in mitochondria, *Eur. J. Biochem.* 10 (1969) pp. 494-500.
63. C. Henchcliffe and M.F. Beal, Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis, *Nat. Clin. Pract. Neurol.* 4 (2008) pp. 600-609.
64. R.C. Henneberry, A. Novelli, J.A. Cox, and P.G. Lysko, Neurotoxicity at the N-methyl-D-aspartate receptor in energy-compromised neurons. An hypothesis for cell death in aging and disease, *Ann. N. Y. Acad. Sci.* 568 (1989) pp. 225-233.
65. D.R. Hunter and R.A. Haworth, The Ca<sup>2+</sup>-induced membrane transition in mitochondria. I. The protective mechanisms, *Arch. Biochem. Biophys.* 195 (1979) pp. 453-459.
66. D.R. Hunter and R.A. Haworth, The Ca<sup>2+</sup>-induced membrane transition in mitochondria. III. Transitional Ca<sup>2+</sup> release, *Arch. Biochem. Biophys.* 195 (1979) pp. 468-477.

67. P.A. Hyslop, D.B. Hinshaw, W.A. Halsey, Jr., I.U. Schraufstatter, R.D. Sauerheber, R.G. Spragg, J.H. Jackson, and C.G. Cochrane, Mechanisms of oxidant-mediated cell injury. The glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide, *J. Biol. Chem.* 263 (1988) pp. 1665-1675.
68. D. James, P.A. Parone, O. Terradillos, S. Lucken-Ardjomande, S. Montessuit, and J.C. Martinou, Mechanisms of mitochondrial outer membrane permeabilization, *Novartis. Found. Symp.* 287 (2007) pp. 170-176.
69. P. Jenner, Oxidative stress in Parkinson's disease, *Ann. Neurol.* 53 Suppl 3 (2003) p. S26-S36.
70. R.B. Jennings, K.A. Reimer, and C. Steenbergen, Effect of inhibition of the mitochondrial ATPase on net myocardial ATP in total ischemia, *J. Mol. Cell Cardiol.* 23 (1991) pp. 1383-1395.
71. J.D. Johnson, J.G. Mehus, K. Tews, B.I. Milavetz, and D.O. Lambeth, Genetic evidence for the expression of ATP- and GTP-specific succinyl-CoA synthetases in multicellular eucaryotes, *J. Biol. Chem.* 273 (1998) pp. 27580-27586.
72. J.D. Johnson, W.W. Muhonen, and D.O. Lambeth, Characterization of the ATP- and GTP-specific succinyl-CoA synthetases in pigeon. The enzymes incorporate the same alpha-subunit, *J. Biol. Chem.* 273 (1998) pp. 27573-27579.
73. B.N. Kholodenko, Control of mitochondrial oxidative phosphorylation, *J. Theor. Biol.* 107 (1984) pp. 179-188.
74. M. Klingenberg, The ADP and ATP transport in mitochondria and its carrier, *Biochim. Biophys. Acta* 1778 (2008) pp. 1978-2021.
75. M. Klingenberg and H. Rottenberg, Relation between the gradient of the ATP/ADP ratio and the membrane potential across the mitochondrial membrane, *Eur. J. Biochem.* 73 (1977) pp. 125-130.
76. Y.H. Ko, M. Delannoy, J. Hullihen, W. Chiu, and P.L. Pedersen, Mitochondrial ATP synthasome. Cristae-enriched membranes and a multiwell detergent screening assay yield dispersed single complexes containing the ATP synthase and carriers for Pi and ADP/ATP, *J. Biol. Chem.* 278 (2003) pp. 12305-12309.
77. Z. Komary, L. Tretter, and V. Adam-Vizi, H<sub>2</sub>O<sub>2</sub> generation is decreased by calcium in isolated brain mitochondria, *Biochim. Biophys. Acta* 1777 (2008) pp. 800-807.

78. D.O. Lambeth, K.N. Tews, S. Adkins, D. Frohlich, and B.I. Milavetz, Expression of two succinyl-CoA synthetases with different nucleotide specificities in mammalian tissues, *J. Biol. Chem.* 279 (2004) pp. 36621-36624.
79. J.W. Langston, P. Ballard, J.W. Tetrud, and I. Irwin, Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis, *Science* 219 (1983) pp. 979-980.
80. M. Leist, B. Single, A.F. Castoldi, S. Kuhnle, and P. Nicotera, Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis, *J. Exp. Med.* 185 (1997) pp. 1481-1486.
81. A.W. Leung and A.P. Halestrap, Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore, *Biochim. Biophys. Acta* 1777 (2008) pp. 946-952.
82. M.T. Lin and M.F. Beal, Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases, *Nature* 443 (2006) pp. 787-795.
83. C. Little and P.J. O'Brien, Mechanism of peroxide-inactivation of the sulphhydryl enzyme glyceraldehyde-3-phosphate dehydrogenase, *Eur. J. Biochem.* 10 (1969) pp. 533-538.
84. X. Liu, C.N. Kim, J. Yang, R. Jemmerson, and X. Wang, Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c, *Cell* 86 (1996) pp. 147-157.
85. J.P. MacManus and A.M. Buchan, Apoptosis after experimental stroke: fact or fashion?, *J. Neurotrauma* 17 (2000) pp. 899-914.
86. M. Manczak, T.S. Anekonda, E. Henson, B.S. Park, J. Quinn, and P.H. Reddy, Mitochondria are a direct site of A beta accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression, *Hum. Mol. Genet.* 15 (2006) pp. 1437-1449.
87. L. Merlini, A. Angelin, T. Tiepolo, P. Braghetta, P. Sabatelli, A. Zamparelli, A. Ferlini, N.M. Maraldi, P. Bonaldo, and P. Bernardi, Cyclosporin A corrects mitochondrial dysfunction and muscle apoptosis in patients with collagen VI myopathies, *Proc. Natl. Acad. Sci. U. S. A* 105 (2008) pp. 5225-5229.
88. E. Metelkin, I. Goryanin, and O. Demin, Mathematical modeling of mitochondrial adenine nucleotide translocase, *Biophys. J.* 90 (2006) pp. 423-432.
89. Y. Mizuno, S. Matuda, H. Yoshino, H. Mori, N. Hattori, and S. Ikebe, An immunohistochemical study on alpha-ketoglutarate dehydrogenase complex in Parkinson's disease, *Ann. Neurol.* 35 (1994) pp. 204-210.

90. Y. Mizuno, K. Suzuki, and S. Ohta, Postmortem changes in mitochondrial respiratory enzymes in brain and a preliminary observation in Parkinson's disease, *J. Neurol. Sci.* 96 (1990) pp. 49-57.
91. T. Nakagawa, S. Shimizu, T. Watanabe, O. Yamaguchi, K. Otsu, H. Yamagata, H. Inohara, T. Kubo, and Y. Tsujimoto, Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death, *Nature* 434 (2005) pp. 652-658.
92. V.P. Nakka, A. Gusain, S.L. Mehta, and R. Raghbir, Molecular mechanisms of apoptosis in cerebral ischemia: multiple neuroprotective opportunities, *Mol. Neurobiol.* 37 (2008) pp. 7-38.
93. T. Nguyen, M. Oghi, and J.A. Johnson, Delta Protein Kinase C Interacts with the d Subunit of the F1F0 ATPase in Neonatal Cardiac Myocytes Exposed to Hypoxia or Phorbol Ester: IMPLICATIONS FOR F1F0 ATPase REGULATION, *J. Biol. Chem.* 283 (2008) pp. 29831-29840.
94. D.G. Nicholls, Simultaneous Monitoring of Ionophore- and Inhibitor-mediated Plasma and Mitochondrial Membrane Potential Changes in Cultured Neurons, *J. Biol. Chem.* 281 (2006) pp. 14864-14874.
95. D.G. Nicholls, Oxidative stress and energy crises in neuronal dysfunction, *Ann. N. Y. Acad. Sci.* 1147 (2008) pp. 53-60.
96. D.G. Nicholls and S.L. Budd, Mitochondria and neuronal survival, *Physiol Rev.* 80 (2000) pp. 315-360.
97. D.G. Nicholls and I.D. Scott, The regulation of brain mitochondrial calcium-ion transport. The role of ATP in the discrimination between kinetic and membrane-potential-dependent calcium-ion efflux mechanisms, *Biochem. J.* 186 (1980) pp. 833-839.
98. A.L. Nieminen, Apoptosis and necrosis in health and disease: role of mitochondria, *Int. Rev. Cytol.* 224 (2003) pp. 29-55.
99. A. Novelli, J.A. Reilly, P.G. Lysko, and R.C. Henneberry, Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced, *Brain Res.* 451 (1988) pp. 205-212.
100. A. Nunomura, G. Perry, G. Aliev, K. Hirai, A. Takeda, E.K. Balraj, P.K. Jones, H. Ghanbari, T. Wataya, S. Shimohama, S. Chiba, C.S. Atwood, R.B. Petersen, and M.A. Smith, Oxidative damage is the earliest event in Alzheimer disease, *J. Neuropathol. Exp. Neurol.* 60 (2001) pp. 759-767.
101. C.M. O'Reilly, K.E. Fogarty, R.M. Drummond, R.A. Tuft, and J.V. Walsh, Jr., Quantitative analysis of spontaneous mitochondrial depolarizations, *Biophys. J.* 85 (2003) pp. 3350-3357.

102. S. Orrenius, D.H. Burgess, M.B. Hampton, and B. Zhivotovsky, Mitochondria as the focus of apoptosis research, *Cell Death. Differ.* 4 (1997) pp. 427-428.
103. T.A. Out, E. Valetton, and A. Kemp, Jr., Role of the intramitochondrial adenine nucleotides as intermediates in the uncoupler-induced hydrolysis of extramitochondrial ATP, *Biochim. Biophys. Acta* 440 (1976) pp. 697-710.
104. L.C. Park, H. Zhang, K.F. Sheu, N.Y. Calingasan, B.S. Kristal, J.G. Lindsay, and G.E. Gibson, Metabolic impairment induces oxidative stress, compromises inflammatory responses, and inactivates a key mitochondrial enzyme in microglia, *J. Neurochem.* 72 (1999) pp. 1948-1958.
105. W.D. Parker, Jr., J.K. Parks, and R.H. Swerdlow, Complex I deficiency in Parkinson's disease frontal cortex, *Brain Res.* 1189 (2008) pp. 215-218.
106. P.L. Pedersen, Transport ATPases into the year 2008: a brief overview related to types, structures, functions and roles in health and disease, *J. Bioenerg. Biomembr.* 39 (2007) pp. 349-355.
107. D. Pratico, K. Uryu, S. Leight, J.Q. Trojanowski, and V.M. Lee, Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis, *J. Neurosci.* 21 (2001) pp. 4183-4187.
108. J. Pu and M. Karplus, How subunit coupling produces the gamma-subunit rotary motion in F1-ATPase, *Proc. Natl. Acad. Sci. U. S. A* 105 (2008) pp. 1192-1197.
109. M.E. PULLMAN and G.C. MONROY, A NATURALLY OCCURRING INHIBITOR OF MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE, *J. Biol. Chem.* 238 (1963) pp. 3762-3769.
110. A.C. Rego and C.R. Oliveira, Mitochondrial dysfunction and reactive oxygen species in excitotoxicity and apoptosis: implications for the pathogenesis of neurodegenerative diseases, *Neurochem. Res.* 28 (2003) pp. 1563-1574.
111. R. Rizzuto, S. Marchi, M. Bonora, P. Aguiari, A. Bononi, S.D. De, C. Giorgi, S. Leo, A. Rimessi, R. Siviero, E. Zecchini, and P. Pinton, Ca<sup>2+</sup> transfer from the ER to mitochondria: When, how and why, *Biochim. Biophys. Acta* (2009).
112. D.J. Rossi, T. Oshima, and D. Attwell, Glutamate release in severe brain ischaemia is mainly by reversed uptake, *Nature* 403 (2000) pp. 316-321.
113. W. Rouslin and C.W. Broge, Regulation of the mitochondrial adenosine 5'-triphosphatase in situ during ischemia and in vitro in intact and sonicated mitochondria from slow and fast heart-rate hearts, *Arch. Biochem. Biophys.* 280 (1990) pp. 103-111.

114. W. Rouslin and C.W. Broge, Why the mitochondrial ATPase inhibitor IF1 fails to inhibit the mitochondrial ATPase in situ in fast heart-rate mammalian and avian hearts, *Ann. N. Y. Acad. Sci.* 671 (1992) pp. 505-506.
115. W. Rouslin and C.W. Broge, Factors affecting the species-homologous and species-heterologous binding of mitochondrial ATPase inhibitor, IF1, to the mitochondrial ATPase of slow and fast heart-rate hearts, *Arch. Biochem. Biophys.* 303 (1993) pp. 443-450.
116. W. Rouslin, G.D. Frank, and C.W. Broge, Content and binding characteristics of the mitochondrial ATPase inhibitor, IF1, in the tissues of several slow and fast heart-rate homeothermic species and in two poikilotherms, *J. Bioenerg. Biomembr.* 27 (1995) pp. 117-125.
117. J.M. Scanlon and I.J. Reynolds, Effects of oxidants and glutamate receptor activation on mitochondrial membrane potential in rat forebrain neurons, *J. Neurochem.* 71 (1998) pp. 2392-2400.
118. A.H. Schapira, J.M. Cooper, D. Dexter, J.B. Clark, P. Jenner, and C.D. Marsden, Mitochondrial complex I deficiency in Parkinson's disease, *J. Neurochem.* 54 (1990) pp. 823-827.
119. A.C. Schinzel, O. Takeuchi, Z. Huang, J.K. Fisher, Z. Zhou, J. Rubens, C. Hetz, N.N. Danial, M.A. Moskowitz, and S.J. Korsmeyer, Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia, *Proc. Natl. Acad. Sci. U. S. A* 102 (2005) pp. 12005-12010.
120. P.H. Schlesinger and M. Saito, The Bax pore in liposomes, *Biophysics, Cell Death. Differ.* 13 (2006) pp. 1403-1408.
121. I.D. Scott and D.G. Nicholls, Energy transduction in intact synaptosomes. Influence of plasma-membrane depolarization on the respiration and membrane potential of internal mitochondria determined in situ, *Biochem. J.* 186 (1980) pp. 21-33.
122. A.A. Starkov, C. Chinopoulos, and G. Fiskum, Mitochondrial calcium and oxidative stress as mediators of ischemic brain injury, *Cell Calcium* 36 (2004) pp. 257-264.
123. S.A. Susin, N. Zamzami, M. Castedo, T. Hirsch, P. Marchetti, A. Macho, E. Daugas, M. Geuskens, and G. Kroemer, Bcl-2 inhibits the mitochondrial release of an apoptogenic protease, *J. Exp. Med.* 184 (1996) pp. 1331-1341.
124. J.J. Tomashek and W.S. Brusilow, Stoichiometry of Energy Coupling by Proton-Translocating ATPases: A History of Variability, *J. Bioenerg. Biomembr.* 32 (2000) pp. 493-500.

125. L. Tretter and V. Adam-Vizi, Inhibition of Krebs cycle enzymes by hydrogen peroxide: A key role of [alpha]-ketoglutarate dehydrogenase in limiting NADH production under oxidative stress, *J. Neurosci.* 20 (2000) pp. 8972-8979.
126. L. Tretter, C. Chinopoulos, and V. Adam-Vizi, Enhanced depolarization-evoked calcium signal and reduced [ATP]/[ADP] ratio are unrelated events induced by oxidative stress in synaptosomes, *J. Neurochem.* 69 (1997) pp. 2529-2537.
127. Y. Tsujimoto, Apoptosis and necrosis: intracellular ATP level as a determinant for cell death modes, *Cell Death. Differ.* 4 (1997) pp. 429-434.
128. G. Van Loo, X. Saelens, M. Van Gurp, M. MacFarlane, S.J. Martin, and P. Vandenabeele, The role of mitochondrial factors in apoptosis: a Russian roulette with more than one bullet, *Cell Death. Differ.* 9 (2002) pp. 1031-1042.
129. V. Vereczki, E. Martin, R.E. Rosenthal, P.R. Hof, G.E. Hoffman, and G. Fiskum, Normoxic resuscitation after cardiac arrest protects against hippocampal oxidative stress, metabolic dysfunction, and neuronal death, *J. Cereb. Blood Flow Metab* 26 (2006) pp. 821-835.
130. A.M. Verhagen, P.G. Ekert, M. Pakusch, J. Silke, L.M. Connolly, G.E. Reid, R.L. Moritz, R.J. Simpson, and D.L. Vaux, Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins, *Cell* 102 (2000) pp. 43-53.
131. S. Vesce, M.B. Jekabsons, L.I. Johnson-Cadwell, and D.G. Nicholls, Acute glutathione depletion restricts mitochondrial ATP export in cerebellar granule neurons, *J. Biol. Chem.* 280 (2005) pp. 38720-38728.
132. P.V. Vignais, Molecular and physiological aspects of adenine nucleotide transport in mitochondria, *Biochim. Biophys. Acta* 456 (1976) pp. 1-38.
133. A.D. Vinogradov, Steady-state and pre-steady-state kinetics of the mitochondrial F(1)F(o) ATPase: is ATP synthase a reversible molecular machine?, *J. Exp. Biol.* 203 (2000) pp. 41-49.
134. C. Volbracht, M. Leist, and P. Nicotera, ATP controls neuronal apoptosis triggered by microtubule breakdown or potassium deprivation, *Mol. Med.* 5 (1999) pp. 477-489.
135. P. Wang and J. Heitman, The cyclophilins, *Genome Biol.* 6 (2005) p. 226.
136. N. Zamzami and G. Kroemer, The mitochondrion in apoptosis: how Pandora's box opens, *Nat. Rev. Mol. Cell Biol.* 2 (2001) pp. 67-71.

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137. J. Zhang, G. Perry, M.A. Smith, D. Robertson, S.J. Olson, D.G. Graham, and T.J. Montine, Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons, *Am. J. Pathol.* 154 (1999) pp. 1423-1429.
138. Y. Zhang, O. Marcillat, C. Giulivi, L. Ernster, and K.J. Davies, The oxidative inactivation of mitochondrial electron transport chain components and ATPase, *J. Biol. Chem.* 265 (1990) pp. 16330-16336.

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