Proton leak through the UCPs and ANT carriers and beyond: A breath for the electron transport chain

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Abstract

Mitochondria produce heat as a result of an ineffective H^+ cycling of mitochondria respiration across the inner mitochondrial membrane (IMM). This event present in all mitochondria, known as proton leak, can decrease protonmotive force (Δp) and restore mitochondrial respiration by partially uncoupling the substrate oxidation from the ADP phosphorylation. During impaired conditions of ATP generation with F_1F_O-ATPase, the Δp increases and IMM is hyperpolarized. In this bioenergetic state, the respiratory complexes support H^+ transport until the membrane potential stops the H^+ pump activity. Consequently, the electron transfer is stalled and the reduced form of electron carriers of the respiratory chain can generate $O_2{\cdot}^-$ triggering the cascade of ROS formation and oxidative stress. The physiological function to attenuate the production of $O_2{\cdot}^-$ by Δp dissipation can be attributed to the proton leak supported by the translocases of IMM.

Keywords

proton leak; proton-motive force; electron transport chain; reactive oxygen species; proton conductance; mitochondria

Abbreviations

Δp, proton-motive force; $\Delta\psi$, membrane potential; ETC, electron transport chain; $O_2{\cdot}^-$, superoxide anion; ROS, reactive oxygen species; IMM, inner mitochondrial membrane; J_{H^+}, proton flux; G_{H^+}, proton conductance of the inner mitochondrial membrane; ANT, adenine nucleotide translocase; UCP, uncoupling protein; PUFA, polyunsaturated fatty acids; BAT, brown adipose tissue.

1. Introduction

The mitochondria in eukaryotic organisms accomplish their function by creating energy in the form of ATP, the universal biological energy currency while consuming oxygen. In mitochondria, the reducing equivalents are transferred, during the tricarboxylic acid cycle, pyruvate oxidation, fatty acid and amino acid catabolism to the cofactors NAD^+ and FAD^+. The reduced cofactors, *i.e.*, NADH and $FADH_2$, are oxidized with mitochondrial respiration and electrons are transferred through the respiratory chain to the final acceptor, O_2. The movement of electrons through the carriers in the electron transport chain (ETC) is led by a reduction potential that increases gradually creating a negative $\Delta G°$ exploited by the respiratory complexes to transfer H^+ from the matrix (negative side) to the space existing between the inner and outer mitochondrial membranes (positive side) [1,2]. The catalytic activity of H^+ pumps of the ETC (complex I, complex III and complex IV) forms an electrochemical gradient of H^+ ($\Delta\mu_{H^+}$), which in terms of proton-motive force (pmf or Δp) is equal in voltage units at $-\Delta\mu_{H^+}/F$ (with F the Faraday constant). The Δp in mammalian mitochondria consists mostly of an electrical gradient (transmembrane potential, $\Delta\psi$) and a small part of a chemical gradient (transmembrane pH difference, ΔpH) [3,4]. The Δp is the main energy source in mitochondria to guide the re-entry of H^+ into the matrix for the synthesis of ATP through the F_1F_O-ATPase and to maintain the ionic homeostasis of metabolites supporting the activity of the inner mitochondrial membrane (IMM) carrier proteins. In the chemiosmotic system, the energy released by oxidation reactions of substrates is coupled to mitochondrial ATP synthesis in a biological process known as oxidative phosphorylation (OXPHOS) [5] (Fig. 1).

The OXPHOS mechanism can be depicted as a circuit in which the proton flux (J_{H^+}) is comparable to the current flowing in an electrical circuit powered by the respiratory complexes that constitute the electrical "chargers". The F_1F_O-ATPase serves as an engine that produces ATP, the membrane potential as the "battery", whereas the circuit resistance includes every step of H^+ translocation through the bilayer of IMM [6]. In an "ideal" situation the Δp is coupled entirely to mitochondrial ATP synthesis. However, the biology of mitochondria provides that Δp retains a physiological mild uncoupling of OXPHOS. This is handled with the H^+ conductance through the IMM (G_{H^+}) that sustains the Δp dissipation responsible for driving the electrons transport and respiration by the ETC in the absence of ATP synthesis [7–9] (Fig. 1).

All mitochondria possess a proton leak through the IMM whose identity and function are still not fully understood [10–13]. Proton leak is a particular type of thermogenic process. The key physiological activities of other various electrogenic conductances of the IMM involved in mitochondrial uncoupling include the control of mitochondrial-regulated cell death by the permeability transition pore (PTP) phenomenon [14]. The endogenous proton leak is highly dependent on the Δp decreasing in state III (active respiration). On the contrary, Δp increases in state IV (respiratory status of rest or controlled) when the mitochondria supplied with substrates convert all added ADP to ATP [15,16]. Therefore, the effective G_{H^+} may sustain mitochondrial respiration in state IV acting as an escape route for H^+ when the Δp increases [10,12]. In state IV, with ETC overloaded by stalled electrons, would increase the risk of superoxide anion ($O_2\cdot^-$) formation in Complexes I and III [12]. Moreover, other powerful oxidant reactive oxygen species (ROS) such as hydroxyl radical (·OH), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^-$) can be obtained from $O_2\cdot^-$ [17].

The production of ROS in mitochondria depends entirely on the state of coupling of OXPHOS. Conditions that lead to obtaining a low rate of electron transfer [18] can increase ROS generation since a prolonged reduced state of the respiratory carriers causes the electron leak [19]. The mild uncoupling of OXPHOS, decreasing the value of the Δp as a consequence of raising the G_{H^+}, stimulates respiration and might reduce the formation of ROS [20,21].

2. Proton leak: fact or artefact

The proton leak causes an uncoupling between the oxidation of the substrate and the phosphorylation of ADP by allowing H^+ of Δp to return to the matrix. Proton leak in mitochondria is demonstrated with Δp increase in the presence of the F_1F_O-ATPase inhibitor oligomycin [13]. Moreover, mitochondrial translocation of monovalent cations and anions by simple diffusion in the absence of energized IMM is the factor that affects the passive osmotic swelling of mitochondria driven by a pH-dependent mechanism of ionophores (Fig. 2). Conversely, the phenomenon is counteracted by proton leak events [22–24]. As already deduced by Krishnamoorthy and Hinkle [18], the H^+ or OH^- flux through the IMM shows non-linear progress as $\Delta\psi$ varies, whereas H^+ or OH^- flow across the membranes is controlled by pH following Fick's Law [25]. Therefore, proton leak is directly proportional to ΔpH [26]. Otherwise, this non-specific membrane permeability to H^+ at high values of Δp violates Ohm's Law. Indeed, the non-ohmic proton leak is revealed by an exponential behaviour of the current-to-voltage (*I/V*) ratio (Fig. 3) [6].

The nonlinear curve, shown in Figure 3, may suggest that the permeability coefficient increases with the membrane voltage, $\Delta\psi$, denoting a non-constant resistance for the H^+ flux. The relative permeability to H^+ of IMM is a function of the electrical component of Δp.

An alternative interpretation to explain the H^+ permeability through IMM (as a function of the Δp) was proposed by Pietrobon [27] suggesting the phenomenon known as "intrinsic uncoupling" or "slip" of the mitochondrial H^+ pumps in which the H^+ permeability coefficient remains constant, but there is a decrease in the stoichiometric ratio H^+/e^- in ETC at high values of Δp [27–29]. Reducing a half-molecule of oxygen, the cytochrome *c* oxidase (Complex IV) couples to each electron transferred to the binuclear haem a_3/Cu_B the pumping of H^+ from the negative side to the positive side of the IMM. The stoichiometry becomes 1:1 for the ratio H^+/e^-. In addition, a "chemical" transport of H^+ by Complex IV from the matrix to *intracristae* space is responsible for the $\Delta\psi$ generation sustained by H_2O production [30,31]. During the intrinsic uncoupling in Complex IV, the electrons can reduce oxygen to H_2O bypassing the haem "*a*" and decoupling H^+ pump to the positive side of the IMM [28]. In this situation, the ratio H^+/e^- decreases since H^+ flow is not driven by the redox potential of the respiratory chain [29,32]. The slip of OXPHOS was observed only in Complex IV but not in Complex I or Complex III. A fundamental possibility of slip is also assumed for Complex III decoupled by DCCD [33] or by cyclic transport of H^+ within the complex sustained with the "rescue pathways" of α,ω-dioic acids to protect against ROS generated in mitochondria [34].

The existence of proton leak events in mitochondria supports different functions: *i*) thermogenesis, a way for the avoidance of dielectric breakdown of IMM at excessive Δp; *ii*) the improved capacity to regulate the oxidative energy metabolism; *iii*) the ability to continue mitochondrial catabolism when the cellular ATP demand is low maintaining a high ratio of NAD^+/NADH suitable for the catabolism; *iv*) regulation of body mass; *v*) attenuation of ROS production [7,35].

The proton leak occurs in endothermic cells as well as in ectothermic cells. Therefore, non-shivering thermogenesis is a mechanism in which mitochondrial respiration is exploited to produce heat without synthesizing ATP through an inducible H^+ conductance sustained by uncoupling protein-1 (UCP1) in brown adipose tissue (BAT) [9,36,37]. However, non-shivering thermogenesis also dwells in other organs of mammals and birds, and in particular in skeletal muscles [38]. The proton leak appears as a "drain" of energy in the mitochondria of many different species including mammals, reptiles, amphibians and molluscs. The true function(s) seems to be so important for those living organisms that are ready to pay the high price of energy dissipation to afford the proton leak [7]. These considerations suggest that the energy cost supported by the mitochondria in this futile cycle of H^+ must be counterbalanced by a high benefit for the cells that interest a great variety of organisms (endotherms and ectotherms). Among the proposed functions, it is also counted the

attenuation of ROS production that is associated with ageing and protection against damage to cellular components. This key role seems to justify the energy cost imposed by the proton leak to the living organism [7]. The loss of energy, attributed to the dissipation of the Δp, is probably necessary for mitochondrial biology to contain oxidative stress [6].

3. Basal and inducible proton leak

The proton leak is thought to be the result of two processes: basal H^+ conductance, which is not regulated, and an inducible H^+ conductance catalyzed by regulated membrane proteins of the IMM [11,39–42]. The physiological relevance of the "basal proton leak" in mammals participates significantly in the basal metabolic rate for the thermogenesis of the body [13]. The basal proton leak is attributed to the G_{H^+} across the lipid bilayer and it has been suggested that the FAs composition of the membrane can modulate it. IMM with a high level of unsaturation index and n-3 polyunsaturated fatty acids (PUFA) and a low level of linoleic acid (18:2 n-6) has a significant proton leak. Indeed, docosahexaenoic acid (22:6 n-3) is correlated with a high H^+ conductance. Therefore, one might expect that n-3 PUFAs are responsible for the increased permeability of the lipid bilayer [43]. However, it cannot be excluded that this effect is additionally the result of peroxidation of n-3 FAs and/or the action of products of their decomposition, which act as powerful activators of the anionic carriers [44]. The increase of the proton leak in membranes rich in PUFAs [45] could be also correlated indirectly with an elevated mitochondrial metabolism that requires more fluid membranes to ensure the appropriate catalytic activity of membrane proteins. The relationship between the presence of PUFAs and the raising of the H^+ conductance would correspond to a basal proton leak assigned to adenine nucleotide translocase (ANT) [39].

The loss of H^+ through the membrane, at the interface between proteins and lipids, may be responsible for most of the basal proton leak [13]. It was calculated that more than half of H^+ conductance is dependent on the abundance of membrane-embedded proteins of IMM, specifically, anion carriers of the family SLC25 as ANT [39,46] and UCP1 in BAT [40]. Genetic manipulation of the amount of ANT embedded in the IMM causes a substantial change in the G_{H^+} [39]. The basal H^+ conductance may be an inevitable consequence of the structure and abundance of ANT although other anionic carriers can participate in the basal proton leak. Since ANT is the most abundant translocase in the IMM, the role of other carriers may be irrelevant [39]. Contrariwise, in BAT mitochondria, the UCP1 is expressed at equal concentrations of ANT. Studies conducted in conditions of the limited presence of endogenous FAs or with inhibitors of UCP1 and ANT, as well as in conditions with UCP1 knockout, have led Parker and colleagues to consider the UCP1 implicated in basal proton leak [40]. However, not all proteins of the IMM are implicated in basal proton leak. The nicotinamide nucleotide transhydrogenase which is up to 2% of total mitochondrial proteins does not affect the basal proton leak. Although it is not exhaustive as absolute proof, available results suggest that the basal H^+ conductance is one way only perpetrated by members of the family of mitochondrial anion carriers and not by other proteins of the IMM. The idea is that the most significant contribution comes from ANT and UCP1 [40].

On the contrary, the inducible proton leak is dependent on the activation of ANT [40], UCPs [9,13,37] or the phosphate carrier [47], the aspartate/glutamate carrier [48], and the dicarboxylate carrier [49]. In brown/beige fat, the mitochondrial proton leak and thermogenesis are verified to be caused by UCP1, whereas the inducible proton leak in extra brown fat tissues was not easily detectable as H^+ conductance activity carrier(s)-dependent [50].

The turnover of ANT, as demonstrated in the presence and absence of a potent inhibitor carboxyatractylate (CAT), is responsible for the CAT-sensitive inducible H^+ conductance that is catalysed by the translocase in

the presence of FAs, AMP or alkenals [11,39,51]. The proton leak catalyzed by ANT can be inhibited not only by classical inhibitors (bongkrekic acid and CAT) but also by ATP, ADP and the GDP. The latter may partly contribute to inhibiting the H^+ conductance binding weakly to ANT in a non-competitive site of adenine substrates without hindering their transport [11]. The molecular identity of the transport protein(s) driving the thermogenic proton leak across the IMM of mitochondria in muscle tissues remained enigmatic for decades, even though proton leak in brown/beige fat is a crucial component of mitochondrial physiology in non-shivering thermogenesis [50]. Recently, studies show that the mitochondria of extra adipose tissues respond to an FA-induced proton leak mediated by ANT [52] and the molecular mechanism generating the H^+ current is similar to the proton leak of UCP1. Proton leak negatively regulated by ADP/ATP exchange via ANT is reliant on cellular control of ATP synthesis and conseistently, cellular ATP requirement may be used to dynamically control proton leak and mitochondrial uncoupling. [51,53,54]. However, ANT is also considered the main component of low conductance supported by PTP [55]. PTP is sensitive to FAs and uncouples mitochondria by H^+ flow through the IMM [56]. ANT might cause mitochondrial uncoupling by proton leak, (non)selective PTP, or both.

The archetypal uncoupling protein, UCP1 [36], with its abundance in BAT, carries out the physiological role in non-shivering thermogenesis importing H^+ or, in some models, transport of FA anions from the inner to the outer leaflet of the IMM [57]. UCP1 is activated by FAs and inhibited with nucleotides [58]. Therefore, the proton leak through the IMM of BAT is primarily physiologically regulated by GDP or by free FAs in the opposite way. Indeed, the UCP1-dependent proton conductance is physiologically activated by FAs that are released by intracellular triacylglycerol under β-adrenergic stimulation in response to cold, or strongly inhibits by the purine nucleotides [59–61]. UCP1 has four different states of conductance and depends on the presence of UCP1 regulatory molecules [37]. The absence of purine nucleotides promotes a state of catalytic activity of UCP1 that is greatly improved by the presence of FAs. There is some debate in the literature on the mechanism related to the role of FAs to induce transport activity since UCP1 can also conduct H^+ in their absence [37,62]. Contrariwise, the purine nucleotides inhibit the activity of UCP1 both experimentally and physiologically [37]. Interestingly, UCP1 is homologous to the ANT whose structure had previously been found [63] and six predicted transmembrane helixes can be arranged into three homologous repeats of two helixes each. The molecular and structural characterization of UCP1 contributes to the elucidation of the mechanistic grounds of its purine nucleotide inhibition. However, one of the most disputed issues in the field of bioenergetics is how UCP1 supports H^+ transport in the presence of free long-chain FAs [64]. The increasing interest in human metabolic disorders related to obesity, including type 2 diabetes and fatty liver disease, has prompted research to understand the mechanisms of non-shivering adaptive thermogenesis [65].

The common criterion for H^+ transport by UCP1 and ANT considers the important role of long-chain FAs (lcFAs) containing more than 12 carbon atoms. In the presence of lcFAs, UCP1 acts as an H^+ uniporter and lcFAs are lodged within UCP1. The hydrophobic tails of lcFAs establish hydrophobic interactions with UCP1 acting as a cofactor for H^+ transport [66]. Indeed, a single lcFA can guide H^+ transport via UCP1 and facilitate conformation changes between *c*- and *m*-state. The protonatable headgroup of lcFA serves as a missing "stepping stone" for H^+ translocation via UCP1 [67].

Moreover, elevated levels of calcium uptake in mitochondria by mitochondrial calcium uniporter (MCU) stimulate the Krebs cycle and supply more protons, promoting uncoupled respiration and acting as a thermogenic uniporter. Upon adrenergic stimulation, MCU recruits UCP1 through the essential MCU regulator (EMRE) to form an MCU-EMRE-UCP1 complex. The recent discovery of a "thermoporter" brings an enhanced H^+ supply for UCP1 operation in the thermogenesis of brown and beige adipose tissue [68].

In contrast to UCP1, FAs do not induce the *c*–*m* conformational change in ANT. Conversely, conformational change happens during the adenine nucleotides translocation mechanism [69], whereas an increase in the ANT-

mediated H^+ translocation action is induced by FAs [50,54,70]. LcFAs must bind to the positive side of the IMM in order to trigger the H^+ conductance via ANT, and ANT can be in either the *c*- or *m*-state to drive the proton leak. Moreover, lcFAs anion bound to ANT may induce a mild conformational change allowing H^+ to move via a narrow translocation pathway of ANT [52].

Noteworthy, DNP, FCCP, SF6847, and BAM15, which are mitochondrial uncouplers that induce pharmacological proton leak across the IMM can activate ANT or UCP1 with a protein-independent protonophoric mechanism emulating the physiological FA-induced mitochondrial proton leak [71].

The strategies of how nucleotides block proton leak via UCP1 and ANT differ from how carriers are activated with FAs. Nucleotides are transported by ANT, whereas they are inhibitors of UCP1. In contrast to ANT, whose nucleotide-binding site alternately opens to both sides of the IMM, UCP1 has a nucleotide-binding site located on the cytosolic face of the IMM [64,72]. Purine nucleotide binding on the positive side of IMM blocks the H^+ translocation of UCP1 [64]. The nucleotide antiport and the FA-dependent H^+ translocation in the ANT reveal a close relationship between mitochondrial ATP and heat generation merging two transport modalities that control mitochondrial ATP production or non-shivering thermogenesis in the bioenergetics process [50].

The "new" uncoupling proteins (nUCPs), UCP2 and UCP3, with a widespread distribution in different tissues, are a field of interest in the analysis of variation in H^+ conductance in mitochondria treated under artificial conditions [37]. Then, UCP4 and UCP5 are mitochondrial carriers widely distributed in the brain but perform similar conformational and H^+ transport activities of UCP1‐UCP3 [73]. Due to the possible neuroprotective effects of the UCP-dependent decrease of ROS production in the nervous system, UCP4 and UCP5 might play a significant role to prevent neurological disorders [74]. Due to their ubiquitous expression, UCP2 and UCP3 may be able to mediate mitochondrial uncoupling in tissues other than brown fat. The nUCPs can transport the H^+ under activation by specific agents, whereas the H^+ conductance is inhibited with purine nucleotides [75,76]. nUCPs catalyze an inducible H^+ conductance in the presence of specific activators, which include the products of lipid peroxidation [9]. How occurs the catalysis of proton leak through UCP2 and UCP3 in the presence of physiological concentrations of ATP and ADP in the cell remains to be understood [37]. It is assumed that the inhibition by purine nucleotides is relieved by the FAs as proposed for the UCP1. Nevertheless, there are no results that consider the nUCPs responsible for a fraction of the proton leak in mitochondria. In the absence of UCP2 and UCP3, mitochondria do not show improved coupling status of OXPHOS [77] and nUCPs are not involved in controlling body weight or adaptive non-shivering thermogenesis [78]. However, UCP3 knockout mice have increased mitochondrial respiration coupling in skeletal muscle mitochondria [79]. Endogenous expression of UCP3 has uncoupling activity and its absence may result in increased ROS production [79] as well as a thermogenic response in skeletal muscle induced by MDMA (ecstasy) [80]. In addition to this, UCP3 contributes to the export of mitochondrial FA anions, preventing mitochondrial damage brought on by lipid peroxidation [81].

Thus, it is difficult to distinguish between an inducible proton leak by nUCPs observed experimentally and what occurs in the cell under physiological conditions [52]. The purported mild uncoupling activity of UCP2 has been reassessed highlighting its biochemical role in mitochondrial oxidation of glucose, glutamine exporting out of mitochondria, and the exchange of four-carbon dicarboxylate Krebs cycle intermediate (*e.g.*, oxaloacetate and malate) for phosphate plus an H^+ from opposite sides of the membrane [82]. In cell bioenergetics, UCP2 reveals a novel regulatory mechanism in cellular metabolic demand or substrate utilization. The UCP2 activity may promote the switch of glucose metabolism to fatty acid metabolism controlling the interaction between UCP2 and ANT [83]. Therefore, H^+ conductance or four-carbon metabolite transport via UCP2 may be influenced by ANT [83].

The physiological differences between the UCPs and F_1F_O-ATPase activities during the Δp dissipation are the uncoupling or coupling of respiration and ADP phosphorylation in mitochondria, respectively (Fig. 1) [21,84]. Therefore, if ATP synthesis by F_1F_O-ATPase was dissipated manipulating cellular energy expenditure, the result would be a mechanism involving direct H^+ recycling to override respiratory control reflecting an UCP-independent thermogenic mechanism based on dissipative hydrolysis of ATP in beige and brown adipose tissue [41]. The suggestion for this bioenergetic phenomenon of non-shivering thermogenesis is attributed to a futile creatine cycle [42]. Mitochondrial phosphocreatine(PCr)/creatine (Cr) circuit is sustained by mitochondrial creatine kinase using mitochondrial ATP in the interconversion of Cr to PCr and liberation of ADP. Contrariwise, a phosphatase might replenish the Cr pool by hydrolyzing PCr. The substrate ATP and the product ADP of creatine cycle are exchanged by ANT increasing the rate of mitochondrial respiration driven by ATP synthesis of F_1F_O-ATPase [42]. Therefore, substrate oxidation during mitochondrial respiration driven by ATP expenditure by futile creatine cycle cause a noncanonical UCP1-independent, but ATP-dependent, non-shivering thermogenesis. This process can counter obesity and glucose dysregulation in pre-clinical models [85–87].

Generally, the inducible proton leak can be alleviated by the addition of bovine serum albumin, which removes FAs and derivatives of reactive alkenals considered endogenous activators of anion carriers of IMM [46]. The endogenous activation of G_{H^+} seems to be directly proportional to the energy state of the IMM and the effect is not dependent on the redox state of ETC but dependent on the $\Delta\psi$. The $\Delta\psi$ could change the conformation of the anion carriers by exposing the binding sites to the activator molecules. The IMM energization dependent on mitochondrial uncoupling, whatever the mechanism involved or the activators who participate, could have its importance for the cell because it limits the Δp during the state IV and as a consequence decreases ROS production [11].

4. Proton leak: a biological formula of prevention against ROS

The formation of superoxide anion depends on the redox potential of the electron donor (respiratory carriers), the concentration of O_2 (the acceptor) and the second-order rate constant for the reaction between them. The standard reduction potential ($E°$) to transfer an electron to O_2 to form $O_2{\cdot}^-$ is -160 mV [88]. By considering $O_2{\cdot}^-$ pK_a value of 4.7 [88], the $E°$ does not vary in the range of physiological pH of living organisms [89]. Since the reduction potential (E) is determined as the product of $E°$ and the ratio $[O_2]/[O_2{\cdot}^-]$, according to possible $O_2{\cdot}^-$ concentrations that might be obtained in the matrix by assuming a low $[O_2]$ of 1 μM, which is enough lower than the 3-30 μM range measured *in vivo*, mitochondria can thermodynamically support the reduction of O_2 to $O_2{\cdot}^-$ [28,89].

The sites of $O_2{\cdot}^-$ formation in the ETC are Complexes I or III, especially in resting conditions with decreased ATP production, slow respiration, high ratio of NADH/NAD^+, and high concentration of reduced coenzyme Q (QH_2) associated with high Δp [90]. Under conditions of low energy demand, the accumulation of NADH in the mitochondria creates a fully reduced FMN in Complex I and consequently the formation of $O_2{\cdot}^-$ [89,91]. Seo and colleagues showed in mammalian mitochondria, independently of the overexpression of NADH dehydrogenase of *Saccharomyces cerevisiae*, an NADH/NAD^+ ratio associated with reduced production of $O_2{\cdot}^-$ [92]. Complex I can generate $O_2{\cdot}^-$ during reverse electron transport that occurs if the electron flow towards O_2 reduction in Complex IV is blocked and there is a high QH_2 pool and Δp [93–96]. In this situation, the electrons of QH_2 are driven by the thermodynamic strength of the Δp to return to Complex I where the production of $O_2{\cdot}^-$ is extremely powerful. However, it can be abolished by decreasing the $\Delta\psi$ [89,97,98]. This conclusion is based on observations that the addition of uncoupling agents, which reduce the Δp, decreases the rate of production of $O_2{\cdot}^-$, although it is the collapse of ΔpH that limits the ROS generation in Complex I [98].

Mitochondria, ensuring for themselves the proton leak, can dissipate the H^+ gradient. Consequently, the H^+ pump activity of respiratory complexes can couple the H^+ uptake to the electrons flux through the ETC decreasing the possibility of $O_2{\cdot}^-$ formation. Otherwise in Complex III, the QH_2 pool is not sufficient to generate $O_2{\cdot}^-$ appreciably to the Q_o site (site of oxidation of ubiquinol) if the Q_i site (site of reduction of ubiquinone) is not inhibited with antimycin A [90].

The rate of $O_2{\cdot}^-$ generation can be very slow if an electron released by "candidate transporter" of the ETC is too far away from O_2. In the biological system, the transfer of electrons is supported by the existence of electron tunnels with maximum distances between donor and acceptor in the range of 14 Å [99,100]. The production of $O_2{\cdot}^-$ anion-sensitive to mild uncoupling can occur only by accessing the sites in which the electrons can be lost and received by O_2. Distances greater than those allowed for a fast transfer between O_2 and respiratory carriers can minimize the formation of ROS [89]. The $O_2{\cdot}^-$ is impermeable to the IMM and has a very short life-time being rapidly converted to H_2O_2 by Mn-superoxide dismutase (Mn-SOD or SOD-2). The H_2O_2 formed is degraded by the enzymes glutathione peroxidase and peroxiredoxin III to H_2O. The H_2O_2 can react alternately through two chemical reactions: with metal ions (such as Fe^{2+}) in the known Fenton reaction or with another molecule of $O_2{\cdot}^-$ in the Haber-Weiss reaction produces the highly toxic ·OH that, unlike $O_2{\cdot}^-$ and H_2O_2, can extract the first hydrogen atom from a methylene ($-CH_2-$) group of PUFA to start the lipid peroxidation process [9]. The $O_2{\cdot}^-$ and lipid peroxidation products are potent activators of H^+ conductance by UCPs in mitochondria. The idea that the $O_2{\cdot}^-$ activates UCPs arises from the results of lipid peroxidation products such as 4-hydroxy-trans-2-nonenal (HNE) inducing the proton leak through UCPs [9,20,101]. Malingriaux and colleagues suggest that aldehyde does not directly activate UCP1 or UCP2. HNE, on the other hand, significantly increased the membrane conductance mediated by different lcFAs in both UCP-containing and UCP-free membranes [102]. Moreover, the PTP may mediate a portion of the proton leak effect of HNE on brown-fat mitochondria [103,104].

The catalytic activity of UCPs can decrease the ROS concentration within the mitochondria [20,105]. These observations suggest the hypothesis for the functions of the UCPs. OXPHOS mild uncoupling UCPs-dependent decreases the Δp and relieves the production of $O_2{\cdot}^-$. Accordingly, cells are protected from oxidative damage at the cost of a slight lowering of the efficiency of OXPHOS [61,77,101]. The relationship between UCPs and ROS has been demonstrated in both isolated mitochondria and intact cells by the existence of a mild uncoupling UCP-specific during the production of ROS. Indeed, the decrease in $O_2{\cdot}^-$ production is reverted by a GDP-sensitive H^+ conductance [101,105]. Therefore, the proton leak can attenuate the $O_2{\cdot}^-$ generation suggesting a system controlled by a feedback loop in which the ROS induces an H^+ conductance sustained by UCPs able to decrease ROS production [20,106]. Considering the uncoupling function of UCPs and the adverse action on mitochondrial energy production, in mitochondrial biology, the apparent ambiguous nature of UCPs has been selected for the protective effect against oxidative stress exploiting a mild uncoupling of OXPHOS [61].

The Δp has a special role also in the supramolecular organization of respiratory complexes that constitute the system of OXPHOS [107,108]. Indeed, the association and the structural organization in respiratory supercomplex can be modulated by Δp [109,110]. The dissociation of supercomplexes in individual units occurs at high values of Δp [111] causing the $O_2{\cdot}^-$ production sensitive to Δp suggesting a link between the two events [110]. Consistently, supercomplexes can hide auto-oxidizable prosthetic groups preventing their reaction with O_2 [112]. This could be a possible explanation for the role of the supercomplex organization to limit ROS formation [97,107]. Moreover, a direct channelling of substrates in supercomplexes increases the flux of electrons and the respiratory chain remains oxidized [97,112,113]. The proton leak might be a "relief valve" dissipating the Δp slowing the disassembly of supercomplexes and preventing the formation of $O_2{\cdot}^-$.

5. Conclusions

During mitochondrial respiration, a cross-connection between the J_{H^+} and the redox reactions ensures the mitochondrial H^+ circuit coupled to electrons transfer in the ETC. Consistently, the resting state should promote the reduced steady state of respiratory carriers without ATP synthesis. Physiological oxygen consumption in the mitochondria independent of ADP phosphorylation can exploit the non-ohmic H^+ conductance stimulated by Δp values above the inherent state IV. The decrease of protonic backpressure on the respiratory chain is also related to UCPs and ANT induction of proton leak. Proton leak, a key component of mitochondrial energy production, is a feature of mitochondria occurring in all tissues. The dissipation of the excessive H^+ potential by proton leak can prevent the formation of $O_2^{\cdot -}$ by OXPHOS mild uncoupling, thus ensuring a system of prevention of damage caused to ROS production. In IMM the increase of the proton leak is probably a safer way for the welfare of aerobic living organisms.

Figure captions

Figure 1. Proton circuit of the mitochondrial chemiosmotic system. Reduced substrates are oxidized by the respiratory complexes (CI, CII, CIII, and CIV) to create the proton gradient (arrows with solid or dashed lines) dissipated by F_1F_O-ATPase (CV) and by the proton conductance through the IMM and/or the mitochondrial carriers, in particular UCPs (uncoupling proteins) and adenine nucleotide translocase (ANT). Q_{10}, coenzyme Q_{10}; Cyt *c*, cytochrome *c*; IMM, Inner mitochondrial membrane. Figure created with BioRender (BioRender.com).

Figure 2. Conditions for welling of non-respiring mitochondria. Passive osmotic swelling of mitochondria in potassium acetate (KAc) (A) in the presence of valinomycin (Val) plus FCCP or (B) in the presence of nigericin (Nig). The decrease of light-scattering, identify as a decrease of optical density (O.D.) of mitochondria spectrophotometrically measured to 540 nm, is related to mitochondrial swelling depending on the nature of the IMM permeability induced by the ionophores. Figure created with BioRender (BioRender.com).

Figure 3. Relationship current-voltage (*I/V*) for ohmic and non-ohmic circuits. Linear ratio *I/V* that observes Ohm's Law (straight line); exponential curve (dash line) that does not comply with Ohm's Law. The non-ohmic trend is typically observed in mitochondria by proton leak (Figure adapted from **[13]**).

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Figure 1

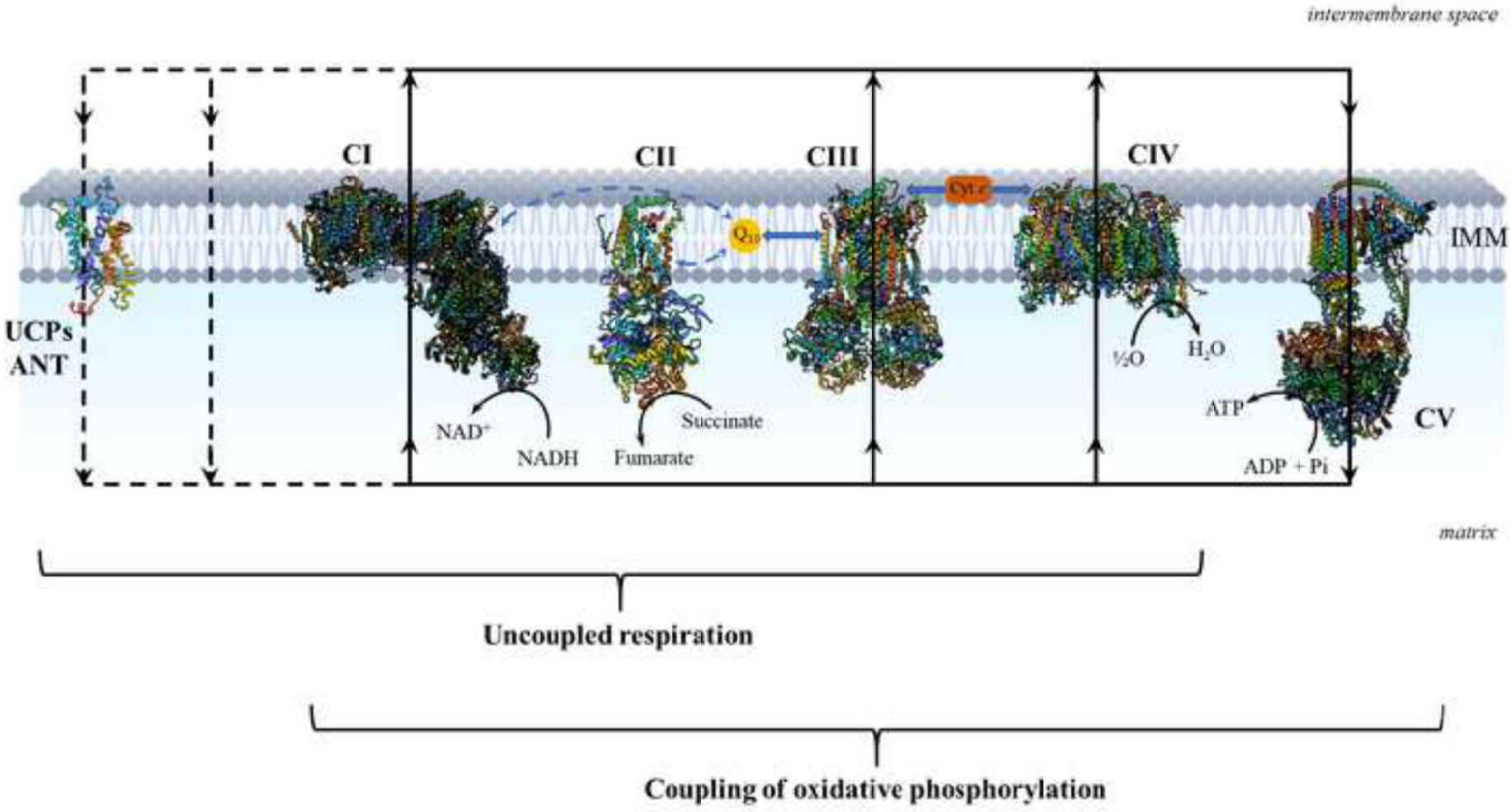

Figure 2

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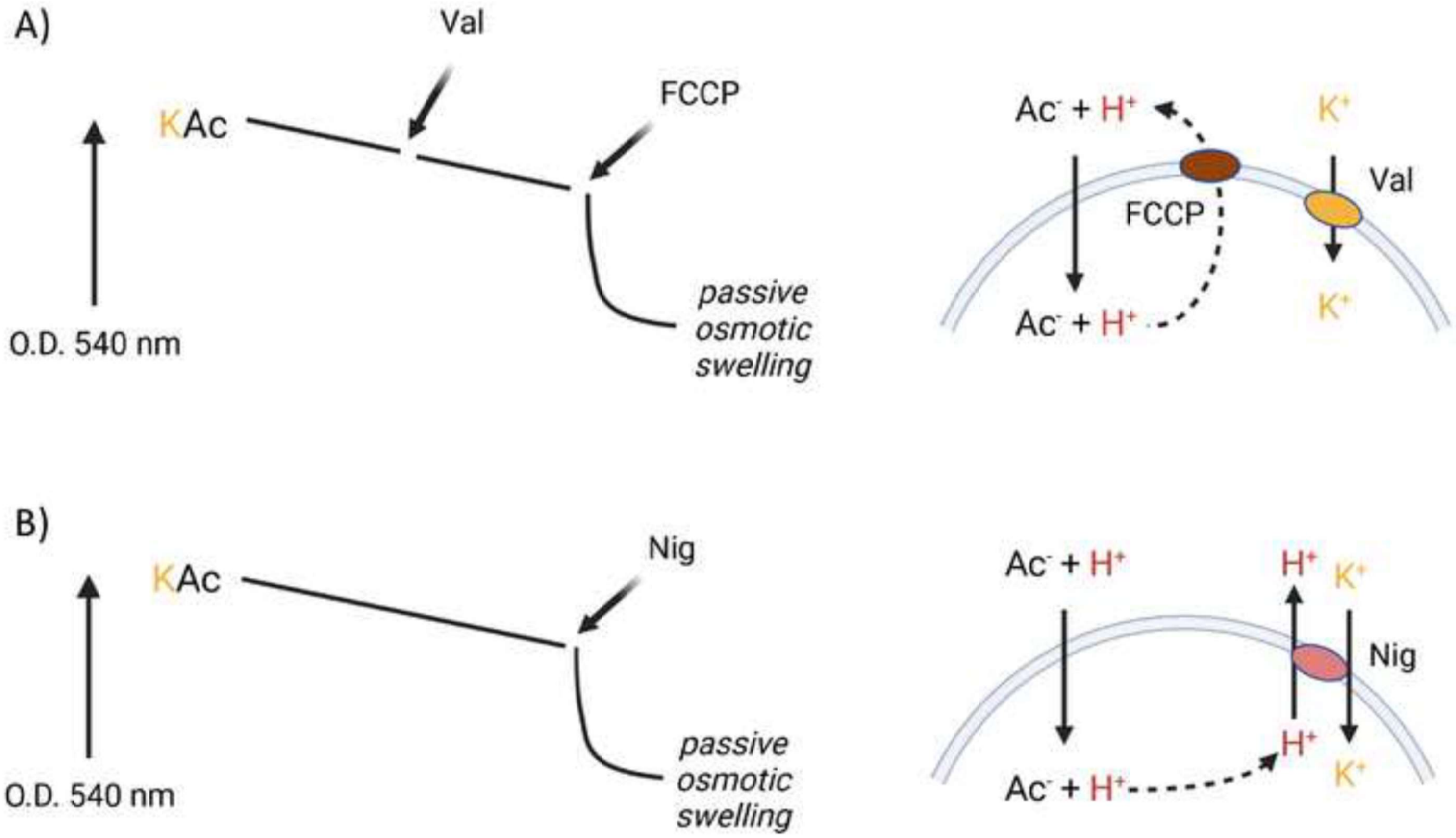

Figure 3

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