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Mitochondria research and neurodegenerative diseases: On the track to understanding the biological world of high complexity

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ABSTRACT

From the simple unicellular eukaryote to the highly complex multicellular organism like Human, mitochondrion emerges as a ubiquitous player to ensure the organism's functionality. It is popularly known as "the powerhouse of the cell" by its key role in ATP generation. However, our understanding of the physiological relevance of mitochondria is being challenged by data obtained in different fields. In this review, a short history of the mitochondria research field is presented, stressing the findings and questions that allowed the knowledge advances, and put mitochondrion as the main player of safeguarding organism life as well as a key to solve the puzzle of the neurodegenerative diseases.

1. Introduction

As a ubiquitous structure of *eukaryotic* cells, the concept of mitochondria emerged from the systematic observation of cells by optical microscopy performed by Richard Altmann at the end of the nineteenth century (Altmann, 1894). Guided by the previous description of intracellular structures reported by others, this researcher detected within nearly all cells branched strings of granules that which he designated by "bioblasts". He proposed that they are "elementary organisms" living inside the cells to ensure vital functions. Without any experimental evidence to support it, this idea was forgotten. However, the similarities between mitochondria and bacteria became evident in the first decades of the 20th century. The visionary idea of Altmann was recovered by the endosymbiotic theory, stating that mitochondria were once free-living aerobic (oxygen-using) bacteria that became an organelle of eukaryotic cells by an (endo)symbiotic process (Sagan, 1967; Margulis, 1975). Whether this association involved two prokaryotic organisms of different life domains (one aerobic bacteria and one anaerobic archaeon as host cell) with the subsequent evolution for the eukaryotic cell, or if a eukaryotic host-cell is present from the beginning of the association remains a controversial subject (Lane and Martin, 2010; Burke, 2017). Despite this, everyone agrees that the mitochondrion is an intracellular structure as defining and ubiquitous among eukaryotes as the nucleus itself (Martin and Mentel, 2010). Additionally, we know that mitochondria contain their own genome (mitochondrial DNA, mtDNA), characterized by multiple copies of a closed circular DNA molecule that is replicated and expressed within the organelle system (Shuster et al., 1988). Human mtDNA only contains 37 genes coding for two rRNAs (ribosomal RNA), 22 tRNAs (transfer RNA), and 13 polypeptides, which are integral components of all subunits of enzyme complexes of the oxidative phosphorylation system (Larsson and Clayton, 1995; Turnbull and Rustin, 2016). Nevertheless, the human mitochondrial proteome

Abbreviations: AD, Alzheimer's disease; ADP, adenosine diphosphate; ATP, adenosine triphosphate; FADH2, reduce flavin adenine dinucleotide; FMN, flavin mononucleotide; GTP, guanosine triphosphate; IMM, inner mitochondrial membrane; IMS, intermembrane space; iNOS, inducible nitric oxide synthase; MitoQ, mitoquinone; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mtDNA, mitochondrial DNA; NADH, nicotinamide adenine dinucleotide; OMM, outer mitochondrial membrane; PD, Parkinson's disease; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; rRNAs, ribosomal RNA; SkQ1, plastoquinone; SS, *Szeto-Schiller*; TPP+, triphenylphosphonium cation; tRNAs, transfer RNA; UCP, uncoupling proteins; 6-OHDOPA, 6-hydroxy-dopamine.

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contains approximately 1500 proteins. This fact indicates that the endosymbiotic process involved gene transfer from mitochondria to the host cell nucleus simultaneously with the development of a mitochondria-specific protein import mechanism to ensure the organelle functionality (Neupert, 1997; Gray et al., 1999; Truscott et al., 2003; Dolezal et al., 2006; Turnbull and Rustin, 2016).

Even so, the term "*mitochondrion*" (plural is "*mitochondria*") was coined, in 1898, by Benda that aggregated two Greek words, "*mitos*" (thread) plus "*chondros*" (granules), to describe the intracellular "thread granules" detected by optical microscopy during spermatogenesis (Benda, 1898). In line with Altmann's morphological description of the "bioblasts", Benda was also the first to assign an intrinsic tubular structure to mitochondria. At that time, knowing whether the intracellular structures named "bioblasts" or "mitochondria" were real intracellular structures or artefacts resulting from cell/tissue staining procedures fed the discussion among researchers. In 1899, Leonor Michaelis took the first step to resolve this issue, showing that the redox dye Janus Green B stains specifically the mitochondria within the cells (Michaelis 1899). Despite Michaelis had not associated the mitochondria with the cellular oxidations trigger by oxygen consumption, it was revealed that mitochondria staining with Janus Green B requires oxygen and it is inhibited with cyanide (Lazarow and Cooperstein, 1953). Therefore, Michaelis's work is considered the starting point of a race to discover the role of the mitochondrion in cell metabolism, referred to by some authors as "enzymological" properties of mitochondria (Lenhinger, 1964; Ernster and Schatz, 1981). Thus, understanding on how the dynamics of cytoplasmic granules known as mitochondria are related to cell metabolism raised as the main research's primary purpose.

In 1909, Regaud showed that mitochondria's histological and staining behavior resembled myelin, concluding that they may be "*corps lipoids*". A few years later, Kingsbury stated that the cytoplasmic granules known as mitochondria or chondriosomes, are the "*structural expression of the reducing substances concerned in cellular respiration*" (Kingsbury, 1912). An idea reinforced by Warburg's work, revealing that the cytoplasmic granules, named by him "grana", enhance the activity of iron-containing respiratory enzymes (Warburg, 1913). Using living cells obtained by tissue culture methods, Lewis and Lewis assigned structural plasticity and dynamics to mitochondria showing that mitochondria change in size, shape, and position inside the cell (Lewis and Lewis, 1915). Today, we know that these findings reflect the continuous processes of fusion and fission that ensure mitochondrial control quality. However, at that time, the lack of knowledge about the chemical composition, structure, and behavior of the mitochondrial components did not allow rationalizing the functional relevance of these observations.

Bensley and Hoerr were aware that to achieve the functions of mitochondria, it would be necessary to develop methodologies to fractionate the cells/tissues and isolate the mitochondria. Thus, in 1934, they reported a centrifugation-based procedure to obtain a fraction rich in globular or rod-shaped structures from liver homogenates in saline aqueous solutions (Bensley and Hoerr, 1934). However, this fraction was not useful to study mitochondria morphology and activity relationship since these globular or rod-shaped structures did not stain with Janus Green B dye. Claude and his collaborators developed a procedure to obtain a mitochondria-rich fraction with functional activity, using sucrose solutions to prepare tissues homogenates and combining the differential centrifugation fractionation technique with the enzymatic characterization of the different fractions obtained (Hogeboom et al., 1946). Hogeboom used this strategy in rat liver to establish that the succinoxidase and cytochrome oxidase are localized exclusively in the mitochondria. It is also important to stress that the current standardized protocol resulted from an upgrade of the procedure mentioned above by reducing sucrose concentration to overcome the inhibitory effect of a high sucrose concentration on several enzymes' activity (Hogeboom et al., 1946; Hogeboom et al., 1948). Using this improved procedure, Kennedy and Lenhinger revealed that the reactions related to the citric acid cycle and fatty acids' oxidation are accomplished by mitochondria (Kennedy and Lenhinger, 1949). Additionally, Lenhinger demonstrated the occurrence of ATP (adenosine triphosphate) synthesis (phosphorylation) coupled to the aerobic oxidation of NADH (nicotinamide adenine dinucleotide), concluding that oxidative phosphorylation occurs in mitochondria (Lehninger, 1949). In the same year, Schneider and Potter reported that mitochondria retain most of the "oxalacetic" oxidase activity of rat liver homogenates, highlighting their action in the Krebs citric acid cycle (Schneider and Potter, 1949). In 1951, Green also showed that the so-called *cyclophorase* system, a particulate preparation obtained from the kidney and liver that catalyze the fatty acid and Krebs cycle oxidations, is a glimmer of mitochondrial activity (Green, 1951). Another feature of intact mitochondria was recognized, in 1951, by Kielley and Kielley when they reported the "latency" of the ATPase activity, which is stimulated by agents that may compromise the mitochondrial structure integrity, such as dinitrophenol (Kielley and Kielley, 1951).

Thus, in the middle of the 20th century, it was well known that the enzymes required for the citric acid cycle, fatty acid oxidation, and oxidative phosphorylation are localized in mitochondria, allowing a full description of the biochemical pathways that support the cell energy demand. These experimental data also indicated that the coupling of respiration to phosphorylation requires structural features of the mitochondrion, and a set of functional enzymes. In this way, the mechanism used by mitochondria to couple the metabolites' oxidation to ATP synthesis became the main research challenge in the biochemistry field.

In 1953, Slater was the first to propose a scientific framework for mitochondrial oxidative phosphorylation, delineating a mechanism coupling oxidation to ATP synthesis (Slater, 1953). According to Slater's mechanism, known as the chemical-coupling hypothesis, the redox reactions between two successive members of the respiratory system (A and B) trap energy initially in a chemical complex (A ~ C). Then, C is transferred to phosphate to form a phosphorylated intermediate (C ~ P) with ability to donate its energy to the ultimate formation of ATP, recycling the unknown compound C (Fig. 1). Slater also suggested that the high-energy complex could drive other reactions and serve as a general energy store within mitochondria.

Indeed, Slater's mechanism was based on the Efraim Racker mechanism for substrate-level phosphorylation by the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase. Despite the efforts developed by several researchers, including Lenhninger, the phosphorylated high-energy intermediates of the respiratory carriers have never been identified.

In the mid-1950s, Green's laboratory developed a procedure to obtain, in large-scale, preparations of beef heart mitochondria with a remarkably high degree of stability and purity which were used to resolve the puzzle of the respiratory chain and the phosphorylation system (Crane et al., 1956). It is important to stress that in the mammalian heart, mitochondria are more abundant than in any other

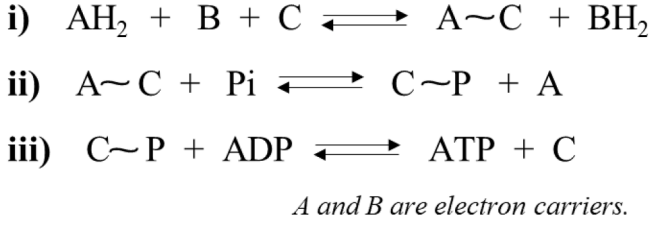

Fig. 1. The chemical couple hypothesis for mitochondrial oxidative phosphorylation proposed by Slater. AH_2 and B are adjacent components of the respiratory chain, C is required for the redox reaction and remains covalently linked to A in an energy-rich compound that can be used to synthesize ATP. A and C must be regenerated from the compound A ~ C to hydrogen-transfer proceed (adapted from Slater 1953).

tissue, accounting for approximately one-third of the cell volume (Bers, 2001). Working with this preparation, Singer and collaborators purified succinate dehydrogenase and demonstrated that the enzyme is a flavoprotein (Singer et al., 1956). Crane and collaborators (Crane et al., 1957) reported that ubiquinone is a redox carrier of the respiratory chain working between the NADH-dehydrogenase or succinate dehydrogenase and the cytochrome system. In 1960, Beinert and Sands discovered a new type of iron-containing redox catalysts that are nonheme iron proteins (Beinert and Sands, 1960; Sands and Beinert, 1960). These catalysts, which were later found to contain iron-sulfur centers as their redox groups, were shown to be components of both succinate and NADH dehydrogenases.

The effort of various groups in Green's laboratory to characterize the protein complexes that catalyze partial reactions of the respiratory chain allowed, in the early 1960 s, the isolation of four different complexes, namely: NADH-ubiquinone reductase (complex I), containing FMN (flavin mononucleotide) and nonheme iron (Hatefi et al., 1961); succinate-ubiquinone reductase (complex II), containing FAD (flavin adenine dinucleotide) and nonheme iron (Ziegler and Doeg, 1962); ubiquinol-cytochrome *c* reductase (complex III), which contains cytochromes *b* and c1, some bound ubiquinone (Hatefi et al., 1962b); and cytochrome *c* oxidase (complex IV), containing cytochrome *a* and cooper (Fowler et al., 1962). In 1962, Hatefi succeeded in reconstituting NADH oxidase and succinoxidase by combining complexes I, III, and IV, and complexes II, III, and IV, respectively, in the presence of cytochrome *c* (Hatefi et al., 1962a). These results gave rise to the concept that the respiratory chain components exist in mitochondria as a fixed assembly ("elementary particles"). Indeed, it was found that cytochrome *c* can form with complex III and complex IV supercomplexes, and the mitochondria contain the cytochromes in near-stoichiometric amounts (Kuboyama et al., 1962).

At the functional level, the works of Chance and Williams have had a strong impact on the mitochondrial research field since they used an oxygen-selective electrode to record polarographically, with high sensibility, the oxygen consumption of mitochondria under different conditions, *i.e.*, substrates, inhibitors, ATP/ADP (ADP, adenosine diphosphate) and uncoupling agents. This methodology was decisive for studying the kinetics of the mitochondrial electron transport activity and its relationship with oxidative phosphorylation (Chance and Williams, 1955; Chance and Williams, 1956) and currently remains available in Hansateck and Oroboros devices. In fact, it made the first quantitative studies possible, characterizing the kinetics of electron-transport catalysts in intact mitochondria and integrated biological systems, including cells and tissue slices. The ATP produced per oxygen atom reduced by the respiratory chain (P/O ratios) activated by NADH-linked substrates or succinate were calculated. Usually, the experimental values were approximated for integers 3 and 2 respectively and rationalized under the chemical-coupling hypothesis framework proposed by Slater (Chance and Williams, 1955; Chance and Williams, 1956).

The oxygen-selective electrode methodology also showed that isolated mitochondria can uptake Ca^{2+} from the suspending medium, and in the presence of phosphate promotes the transient enhancement of the oxygen consumption rate like ADP. The energy-dependent accumulation of calcium and many other divalent cations by respiring mitochondria in the presence of phosphate was quickly confirmed/revealed by other investigators (Deluca and Engstrom, 1961). In 1963, Saris showed that Ca^{+2} uptake is accompanied by a release of protons (Saris, 1963). Moreover, Chance revealed that the mitochondrial Ca^{2+} uptake is driven by the energy of the respiratory chain independently of the phosphorylating system since it is sensitive to uncouplers (*e.g.,* dinitrophenol) and insensitive to oligomycin, an inhibitor of mitochondrial phosphorylation (Chance, 1965). These experiments highlighted the role of mitochondria in the regulation of intracellular fluxes of calcium. Additionally, they also showed the failure of the chemical-coupling hypothesis to explain how mitochondria connect the metabolites oxidation with ATP synthesis. Thus, to understand mitochondrial functionality it is required a new vision that incorporates the available mitochondria architecture data and brings the membrane transport knowledge to the mitochondria research field.

2. Unveiling the interdependence between 3-D architecture and function: a scientific framework to describe the oxidative phosphorylation

With the application of electron microscopy to the study of cells and tissues, during the 1950 s, the first high-resolution micrographs revealing mitochondria as ellipsoid/spherical structures containing more than one membrane system emerged. Sjostrand described the mitochondria as a two membranes system, the outer and the inner membrane, and postulated the existence of a third membrane that separates the matrix into multiple compartments (Sjostrand, 1956). Palade also observed two mitochondrial membranes, proposing that the microscopy structures that seem to separate the mitochondrial matrix into multiple compartments are invaginations of the inner membrane to form folded tubular structures, *i.e.*, *cristae mitochondriales* (Palade, 1952; Palade, 1964). In agreement with Palade's model for mitochondria, Whittaker suggested that mitochondrial cristae are relatively narrow orifices; thereby, they can appear in many micrographs as structures without apparent connection to the inner membrane (Whittaker, 1966). Although to cristae number was early recognized functional relevance, the technology to address the organization and opening size of these "relatively narrows orifices" just became available in the middle of the 1990s of the 20th century. Consequently, its putative functional relevance remained dormant for several decades. Therefore, mitochondria came to be represented as an ellipsoid/spherical structure that combines an outer mitochondrial membrane (OMM) and an inner mitochondrial membrane (IMM) as one continuous closed surface with multiples cristae, creating two distinct compartments: the intermembrane space (IMS), between two membranes, and the mitochondrial matrix, delimited by the IMM. The essential of Palade's model was preserved in the standard model for mitochondria structure, depicted in academic textbooks by cartoons showing the cristae with broad openings to the intermembrane space and relatively long projections to the matrix, like the bellows of an accordion (Fig. 2).

Focused on the emerging ideas about the structural organization and function of properties of cell membranes (Robertson, 1959) that are also evidenced by the 3-D structures of mitochondria described above, Peter Mitchell advanced in 1961 with the *chemiosmotic* hypothesis to solve the puzzling problem of oxidative phosphorylation (Mitchell, 1961). With this hypothesis, Peter Mitchell intended to explain the coupling between oxidation–reduction and phosphorylation without assuming the existence of chemical intermediates common to the oxidation–reduction and phosphorylation pathways. Thus, he moves away from the standard vision proposed by Slater (explained above) to address the challenge of energy transduction in biological systems (*i.e.,* mitochondria, chloroplast, and plasma membrane of bacterial cells) with innovative ideas. The mature version of the *chemiosmotic* theory, published in 1966, is based upon four basic postulates: i) a membrane-located ATPase/synthase system which couples reversibly the translocation of protons across the membrane with ATP synthesis/hydrolysis. In mitochondria, the inwards protons translocation to the matrix is coupled with ATP synthesis, while a proton flows outwards (*i.e.,* from the matrix to intermembrane space) is generated by ATP hydrolysis; ii) the elements of the electron transport (respiratory) chain system exhibit an anisotropic spatial membrane organization that couples the redox reactions with a vectorial proton translocation from one side to another side of the membrane, creating a transmembrane electrochemical gradient of protons. In mitochondria, the elements of the electron transport chain used the energy of redox reactions to pump protons outwards, generating a transmembrane electrical potential (negative inside) and/or pH difference across the IMM; iii) the entry and exit of the essential metabolites

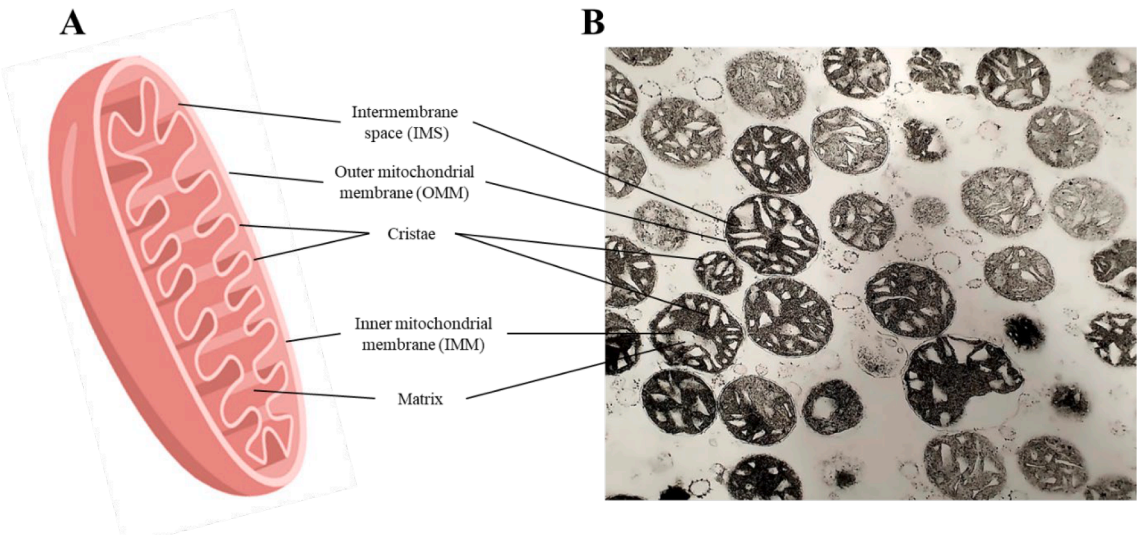

Fig. 2. A. Representation of Palade's mitochondria model, highlighting the outer mitochondrial membrane (OMM), inner mitochondrial membrane (IMM) with its cristae, and the intermembrane space (IMS). B. Electron microscope micrograph of mitochondria isolated from rat liver with similar resolution to those allow to build the model.

are ensured by substrate-specific exchange/diffusion carriers that permit the reversible transmembrane exchange of the anionic metabolites against OH^- and the cationic against H^+ without the collapse of the membrane potential; iv) the specialized coupling membrane (*e.g.,* IMM) contains the systems postulated in i, ii, and iii, *i.e.,* ATPase/synthase system, elements of the oxidation–reduction chain system and the substrate-specific exchange/diffusion carriers, respectively, and has a low permeability to protons as well as to anions and cations in general (Mitchell, 1966). This set of postulates give functional meaning to the available 3-D architecture of mitochondria and allow us to explain the existing data about the oxidation–reduction reactions of the respiratory chain and phosphorylation system (ATPase/synthase) as well as the respiratory parameters obtained from different substrates, selective inhibitors, and uncoupling agents. Additionally, they also allow the design of new experiments intended to test the validity of the hypothesis that energy conservation in the oxidation–reduction chain proceeds by way of a proton gradient across the coupling membrane.

In fact, the experimental data supporting the theory came successively, and they had contributions of a vast number of researchers, as reviewed elsewhere (Hatefi, 1985). However, the known "chloroplast acid bath experiment" pointed out in 1966 by Jagendorf and Uribe was particularly relevant for the general acceptance of chemiosmotic theory (Jagendorf and Uribe, 1966). These researchers obtained ATP synthesis in isolated chloroplasts inducing a transmembrane pH gradient. Thus, in 1978, Peter Mitchell was laureate with Nobel Prize in chemistry by the formulation of the chemiosmotic theory.

Despite the elegance and enormous application scope of Peter Mitchell's proposal, the idea of an electrochemical gradient across a coupling membrane to drive ATP synthesis and other functions was far from the thought of many researchers of that time. For instance, Paul Boyer continued to work on the conformational hypothesis, considering that ATP synthesis was driven by the energy of conformational changes in the respiratory proteins energized by oxidation–reduction reactions. Using ^{32}P as a probe, he even announced the discovery of a catalytic intermediate in ATP formation with a phosphoryl group attached to a histidine residue. However, Paul Boyer's research team quickly concludes that this enzyme is an intermediate in the substrate-level phosphorylation of the citric acid cycle and not a key to oxidative phosphorylation (Boyer et al., 1973).

Considering Peter Mitchell's framework, understanding how energy stored in membrane potential and/or proton gradient generated by the respiratory chain could be used to drive ATP synthesis turns into a challenge. Racker and collaborators ascertained that the synthesis of ATP occurred on the so-called "spheres" referred to as F_1 subunits of the enzyme "FoF_1-ATPase" (Kagawa and Racker, 1966). Considering the available biochemical data, Boyer proposes the "binding change mechanism" for ATP synthesis, suggesting that the energy of the proton gradient is coupled to the rotational movement of catalytic subunits relative to a core of noncatalytic subunits. Consequently, the energy is required to bind ADP and the phosphate to the F_1 subunits enzyme and release ATP from the enzyme (Boyer, 1975; Boyer, 1989). Walker and his co-workers determined the protein subunits' primary structure (amino acid sequences) and established the 3-D structure of the enzyme by crystallography (Abrahams et al., 1994), which allowed the validation of the mechanism proposed by Boyer. The scientific relevance of these findings was fully recognized in 1997 when Paul Boyer and John Walker were laureated with the Nobel Prize in Chemistry.

In fact, the chemiosmotic hypothesis complemented with the binding change mechanism for ATP synthesis has contributed significantly to our understanding of how biological systems convert and store energy. Additionally, it strongly influenced the molecular biology development, especially the protein technology. The main IMM organizational vicissitudes exhibited by the mitochondrial redox chain complexes and FoF_1-ATP synthase, unveiled during the last four decades (Abrahams et al., 1994; Tsukihara et al., 1996; Xia et al., 1997; Iwata et al., 1998; Sun et al., 2005; Efremov et al., 2010; Hunte et al., 2010; Vinothkumar et al., 2014; Sousa et al., 2018; Spikes et al., 2020), support Peter Mitchell vision for mitochondria functionality.

3. Current challenges in mitochondria research – How 3-D architecture fits with function

Mitochondrial bioenergetic functions are essential to cell life and death. Combining high-resolution fluorescent microscopy (including confocal microscopy) with mitochondria-targeted fluorescent dyes allows showing that, in living cells, mitochondria form a branched and long network with a dynamic dependence of the metabolic state and cell-cycle phase (Farkas et al., 1989; Cottet-Rousselle et al., 2011), as illustrated in Fig. 3A. These mitochondrial networks maintain themselves in a dynamic morphology, mainly around the nucleus, by undergoing frequent cycles of division (fission) and merging (fusion) (Twig et al., 2008a; Twig et al., 2008b; Otera and Mihara, 2012; Youle and Bliek, 2012; Chauhan et al., 2014). Indeed, the unveiling of mitochondrial fusion and fission processes is the recognition of the functional

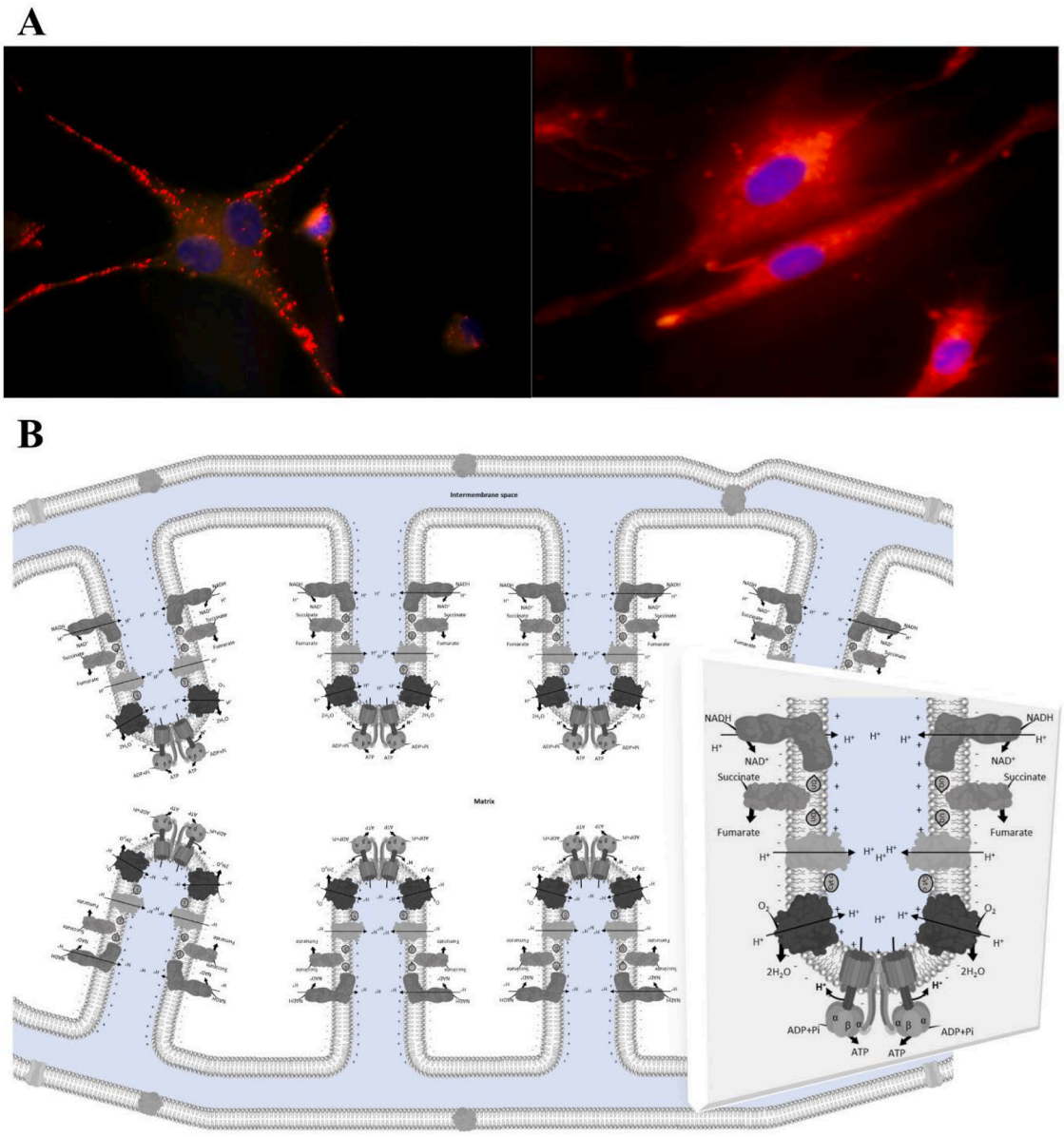

Fig. 3. A. Sertoli cells stained with the mitochondrial probe JC-1 and with Hoechst (color blue nuclei) to show the mitochondrial network in the cytosol. The images were constructed by merging three channels – blue nuclei, red mitochondria polarized, and green mitochondria depolarized. B. Schematic representations of the organization of the oxidative phosphorylation system in the IMM cristae according to the Cryo-Tomography studies, with one mitochondrial cristae magnified to highlight the schematic representation of dimeric organization of the FoF1-ATP synthase and the respiratory chain complexes in the IMM invaginations that form the cristae. *Abbreviations*: cytc, cytochrome *c*; IMM, inner mitochondrial membrane; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolo-carbocyanine iodide; UQ, ubiquinone. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

relevance of the structural plasticity attributed to mitochondria by Lewis and Lewis in 1915 (Lewis and Lewis 1915).

The application of electron microscopic tomography to study mitochondria provides important new insights on organelle 3-D architecture (Perkins et al., 1997; Frey et al., 2002; Frey and Mannella, 2000) that emphasize the need for the development of a new model to represent the functional organization of the organelle and eventually update Peter Mitchell's vision. Electron microscopic cryo-tomography data show that cristae can vary from simple tubular structures to more complex lamellar structures merging with the inner boundary membrane through tubular structures with about 28 nm in diameter (Frey et al., 2002; Frey and Mannella, 2000; Davies et al., 2014). Electron Cryo-Tomography studies of cell and tissues stained with protein-specific fluorescent dyes also revealed that "*ATP synthase is arranged in rows of dimers along highly curved apices of the inner membrane cristae with complexes of the respiratory redox chain located in the membrane areas either side of the rows*" (Dudkina et al., 2010; Davies et al., 2014; Anselmi et al., 2018; Blum et al., 2019) as represented in the Fig. 3B. While the physiological relevance of this oxidative phosphorylation system membrane organization is not fully understood, it is known that FoF_1-ATP synthase dimerization is a crucial player in 3-D inner membrane cristae organization (Anselmi et al., 2018; Blum et al., 2019). Indeed, severe human diseases, including Leigh syndrome, are characterized by significant changes in the 3-D architecture of the mitochondria resulting from genetic alterations that hamper the establishment of FoF_1 ATP synthase dimers (Kucharczyk et al., 2009; Dautant et al., 2018). Experimental evidence also showed that the disruption of FoF_1-ATP synthase dimerization strongly impacts 3-D mitochondrial cristae organization with minor effects on the catalytic activity of the monomeric FoF_1-ATP synthase form (Siegmund et al., 2018; Barca et al., 2018). Despite Cryo-

Tomography allows to obtain images of the 3-D architecture of mitochondria with very high-spatial resolution, it is unable to given information about the temporal scale of morphological changes since the dynamics of biological processes is stopped by cryo-preservation. Super-resolution nanoscopy techniques, including stimulated emission depletion microscopy, are addressing this constraint by using fluorescent mitochondria-tags with improved photostability to detect the mitochondria dynamics during several minutes, recording sequential images with spatial resolution less than 60 nm (Wang et al., 2019; Glancy et al., 2020; Yang et al., 2020).Thus, the mitochondrial fusion and fission processes as well as the dynamic reorganization of mitochondrial cristae (*e.g.*, number, shape, matrix volume versus inner membrane volume) are being revealed in cell culture systems under different metabolic and external stimuli (Wang et al., 2019). The organization of redox complexes of the respiratory system and phosphorylation system, revealed by Cryo-Tomography studies, and cristae dynamics, revealed by super-resolution nanoscopy techniques, are also dependent on a specific pool of IMM lipids, mainly of the cardiolipin content and diversity (Lange et al., 2001; Pfeiffer et al., 2003; Chicco and Sparagna, 2007; Osman et al., 2011; Horvath and Daum, 2013). Cardiolipin is an anionic tetra-acyl phospholipid selected by evolution to optimize functional membrane assembly of redox complexes of the respiratory system, being considered the lipid fingerprint of mitochondria. The mitochondrial dysfunction associated with many human chronic diseases (*e.g.,* Alzheimer's (AD) and Parkinson's (PD) diseases) is characterized not only by the deficit of the respiratory activity system and/or phosphorylation system but also by anomalous cardiolipin profile (Pfeiffer et al., 2003; Chicco and Sparagna, 2007; Petrosillo et al., 2008; Monteiro-Cardoso et al., 2015; Tyurina et al., 2015; Camilleri et al., 2020; Falabella et al., 2021). These data suggest that the 3-D architecture of mitochondria with a particular oxidative phosphorylation system organization in IMM and a particular lipid composition was preserved by evolution not only to allow the coupling between oxidation–reduction and phosphorylation (ATP production) but also to support other fundamental requirements of multicellular life, as it will be exemplified below.

Mammalians and other homeothermic organisms control their body temperature at values that, in general, are higher than the environmental temperature, which requires a controlled process of continuous heat generation (thermogenesis) to counteract heat loss to the environment by thermic difference. The heat generation is related to mitochondrial functionality, and the shivering and non-shivering mechanisms were described as thermogenesis. Shivering thermogenesis is associated with the skeletal muscle tissue that releases significant amounts of heat during contractile activity. The contractile activity of the skeletal muscle is mainly supported by the ATP generated by the intermyofibrillar mitochondria population at the z-bands of the muscle sarcomere thereby, it is dependent on the mitochondrial functionality (Ricquier and Bouillaud, 2000; Solmonson and Mills, 2016; Haman and Blondin, 2017; Vincent et al., 2019). Non-shivering thermogenesis is a generic designation for the heat generated associated with the metabolic activity unrelated to movement (Ricquier and Bouillaud, 2000; Solmonson and Mills, 2016; Nowack et al., 2017). The heat released by the activity of mitochondria under uncoupled conditions, well documented to brown and beige adipose tissues, is particularly relevant. In this mechanism, uncoupling proteins (UCP) play a key role, since they promote the diffusion of protons through the IMM, allowing that all energy of the oxidation of the NADH and $FADH_2$ (reduce flavin adenine dinucleotide) along the respiratory chain can be dissipated as heat rather than to ATP synthesis (Nicholls and Locke, 1984; Ricquier and Bouillaud, 2000; Nowack et al., 2017). Thus, mitochondrial architecture apparently tunned for ATP synthesis serves efficiently another purpose by including a new protein-type (*e.g.*, UCPs) in its IMM. Recent studies also revealed that the temperature around the IMM rises 10 °C above the temperature of the cytosol (37 °C for human cells) when the respiratory chain is fully functional and coupled with ATP synthesis, as detected by temperature-sensitive fluorescent targeted to mitochondria (Chrétien et al., 2018). How mitochondria are able to dissipate such a tremendous amount of heat under coupled conditions, and what is the role of this thermogenesis type for body temperature regulation is remain as open issues. In our understanding, the formulation of any convincing explanatory hypothesis for these questions should be driven by the 3-D architecture of mitochondria with its oxidative phosphorylation system organization in the IMM.

A cornerstone of the highly complex eukaryotic organisms (as humans) is the homeostatic control of the balance between cell proliferation and cell death in tissues, which is required to ensure the organism's good development and physiological performance. Mitochondria are a key player in either cell proliferation or cell death. Cell proliferation requires continuous biosynthetic processes, which are highly dependent on ATP, reduced NADH and $FADH_2$ cofactors, heme prosthetic group, and intermediary metabolites produced in mitochondria (Duchen, 2004; Ajioka et al., 2006; Heikal, 2010; Galluzzi et al., 2012). Cell death by apoptosis (Kerr et al., 1972) is also associated with mitochondria organization and functionality (Kroemer et al., 2007; Vakifahmetoglu-Norberg et al., 2017). Two general mechanisms were used to describe apoptosis, one triggered by external stimuli that activate the cell death receptors on the plasm membrane (extrinsic) and the other by an intrinsic mechanism associated with mitochondrial dysfunctions. In both mechanisms, the release of cytochrome *c* (an electron membrane carrier of the respiratory chain) from the IMM to the cytoplasm is a key event that precedes the activation of the apoptotic caspases cascade. Thus, apoptosis is associated with a profound change in the functional 3-D architecture of mitochondria required for cytochrome *c* release (Igney and Krammer, 2002; Kroemer et al., 2007; Burke, 2017; Vakifahmetoglu-Norberg et al., 2017).

4. Mitochondria and degenerative brain diseases - the case of Alzheimer's and Parkinson's diseases

Degenerative brain diseases that cause progressive and irreversibly brain functions, including memory, cognitive, behavioral, and motor coordination skills, are devastating the lives of millions of people. Currently, 45 million people worldwide suffer from AD and 10 million other neurodegenerative diseases, including PD, without any reliable therapeutic tool to stop or slow their progression (Jagaran and Singh, 2021). Thus, solving the problem of progressive brain degeneration is a scientific challenge with high social and economic relevance.

It is well-known that the loss of functional connectivity between different brain regions, resulting from distorted/disrupted neural networks, is the main macroscopic feature of brain pathologies. At the same time, mitochondrial dysfunction is a cellular hallmark of neurodegeneration, including AD and PD (DeTure and Dickson, 2019; Balestrino and Schapira, 2019). However, the relationship between mitochondria functionality and the brain processes used to generate and coordinate, in space and time, the information flows underlying the cellular networks connectivity remains unclear. Consequently, some investigators consider mitochondrial dysfunction the first cause of the pathological cascade and others as a consequence of the degenerative process, as highlighted in many reviews (Federico et al., 2012; Panchal and Tiwari, 2019; Wang et al., 2019; Wu et al., 2019).

In terms of therapeutic-oriented research, the problem of age-related neurodegeneration has been addressed by a disease-specific pathophysiological vision focused on protein misfolding and genetic vicissitudes specifically connected to each disease (*e.g.,* amyloid-β peptides and tau for AD and alpha-synuclein for PD) (Selkoe, 2004; Soto and Estrada, 2008). Hence, the canonical vision of neurodegenerative diseases recognizes mitochondrial dysfunction as consequence of the degenerative process. However, the research developed for more than two decades under the canonical vision produced disappointing outcomes, since the therapeutics developed to target amyloid-β peptides production/aggregation have failed in clinical trials (Schneider et al.,

2014), and alpha-synuclein-targeted therapies have not been successful either (Brundin et al., 2017; Fields et al., 2019). Thus, the arsenal of available drugs to fight AD and PD remains limited to the first generation of pharmacological molecules approved by their positive impact on specific neural networks. In AD, the pharmacological drugs were rationalized to modulate the cholinergic (acetylcholinesterase inhibitors such as donepezil, galantamine, and rivastigmine) or glutamatergic (memantine) neuronal networks (Butterfield and Pocernich, 2003; Marucci et al., 2021). In PD, they were designed to improve dopaminergic connectivity, increasing dopamine levels working as dopamine precursors (*e.g.,* carbidopa-levodopa) or as inhibitors of dopamine catabolism enzymes (*e.g.,* selegiline, rasagiline, and safinamide that are monoamine oxidase-B inhibitors) (Cezsi and Vecsei, 2017; Tambasco et al., 2018). However, we all know that the therapeutic benefits of these drugs are not sustained in time, and their continuous use has been associated to significant toxic side effects (Jagaran and Singh, 2021).

In recent years the strategies to fight neurodegenerative diseases have been directed towards a vision that considers mitochondrial dysfunction a primary cause of the neurodegenerative process. In defense of this vision, we can assert that: i) mitochondrial dysfunction is an early pathological mark of the neurodegenerative process, and the dysfunctional mitochondria generate large amounts of reactive oxygen species (ROS, mainly at levels of complexes I and III) and drive the cell to the release of pro-inflammatory mediators, leading to oxidative stress and chronic inflammation, other pathological hallmarks of the neurodegeneration (Picca et al., 2017; Grazioli and Pugin, 2018; Picca et al., 2020); ii) intoxications by drugs that work as specific mitochondrial complex I inhibitors (*e.g.,* rotenone, MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) leads to a PD-like neurodegenerative process (Sherer et al., 2003; Richardson et al., 2007); iii) in animal models for neurodegeneration, including AD and PD, the mitochondrial dysfunction is an upstream cellular signal from the aggregation of disease-specific misfolded proteins, preceding the clinical symptoms of each disease (Hashimoto et al., 2003; Melov et al., 2007; Argueti-Ostrovsky et al., 2021; Picca et al., 2021); iv) massive synapse loss precedes neurons death, and the mitochondrial dysfunction is initially detected in the synaptic brain mitochondria population (decline of complex I activity and abnormal cardiolipins profile) as reported for AD-like pathology (Monteiro-Cardoso et al., 2015); v) an imbalance between the dynamic mitochondrial fission and fusion process, mainly resulting from an enhancement of the fission with consequent excessive fragmentation of the mitochondrial network and dysfunctional intracellular distribution, is early detected in animal models of both AD and PD (Bonda et al., 2010; Manczak et al., 2011; Wang et al., 2017; Yang et al., 2021). Accordingly, mitochondria emerge as a promising target to address the neurodegeneration issue, with the putative benefit that a single therapeutic approach can be suitable for multiple common disorders. However, the functional organization of the mitochondrial supports a hub of cell functions that are impaired by neurodegeneration, and the selection of the specific mitochondrial targets that need to be modulated to interrupt (or avoid) the degenerative process remains an unsolved issue.

In the context of neurodegeneration, the first attempts to modulate mitochondrial functionality relied on antioxidants molecules. The main goal of this approach is to prevent the oxidative stress resulting from the overproduction of ROS connected with mitochondrial dysfunction, and the main outcomes obtained in animal models for AD and PD with coenzyme Q10, vitamin E, and lipoic acid are highlighted in Table 1. Overall, positive outcomes in AD and/PD animal models were reported for all antioxidant compounds indicated in Table 1 as well as for many other antioxidants, including the plant polyphenols in formulations prepared with pure compounds or with complex mixtures like polyphenol-rich extracts (Karuppagounder et al., 2009; Ho et al., 2013; Mendes et al., 2018; Tikhonova et al., 2020).

The benefits obtained in animal models sustained that Coenzyme Q10 and lipoic acid were also examined in clinical trials for PD and AD, respectively (Table 2). Formulations of Coenzyme Q10, in a

Table 1
Therapeutic approaches for AD and PD supported by antioxidants (coenzyme Q10, vitamin E, and lipoic acid) or antioxidant molecules engineered to target mitochondria (mitoQ, Mito-Apocynin, SkQ1, and SS-31), highlighting the main outcomes in the indicated animal model used under a specified administration route. Abbreviations: AD, Alzheimer's disease; PD, Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDOPA, 6-hydroxy-dopamine; ATP, Adenosine triphosphate; TPP^{+}, triphenylphosphonium cation; iNOS, inducible nitric oxide synthase; mtDNA, mitochondrial DNA.

Antioxidant molecules	Disease	Animal model/ Administration route	Main outcomes
Coenzyme Q10	AD and diabetes	Diabetic Goto–Kakizaki aged rat model (20-months-old) – Treatment for 7 weeks (injected each 48h)/ Intraperitoneal.	Improved the mitochondrial respiratory control ratio and oxidative phosphorylation efficiency compromised in this animal model (Moreira et al., 2005).
	AD	Transgenic Tg19959 mouse model (1-month-old) – Treatment from 1 to 4 months of age or 1 to 6 months of age/ Oral, chow supplementation.	Decreased brain oxidative stress, Aβ42 levels, plaque burden, and improved cognitive performance of the animal model (Dumont et al., 2011).
	PD	MPTP-induced PD C57BLr6 mouse model (1-year-old) – Treatment for 5 weeks/ Oral, chow supplementation.	Attenuated the MPTP-induced loss of dopamine levels and dopaminergic neurons in the striatal brain region (Beal et al., 1998).
Vitamin E or γ-Cyclodextrin-Vitamin E complex	PD	6-OHDOPA-induced PD in adult Sprague-Dawley rat model – Treatment for 4 weeks (1h before the PD-like toxic stimulus and continued at 2-day intervals)/ Intramuscular.	Protected the brain dopaminergic neurons from 6-OHDOPA toxicity (Roghani and Behzadi, 2001).
	Brain aging	C57BL/6J mice (17-months-old) – Treatment for 24 weeks/ Oral, chow supplementation.	Increased the brain levels of ATP and enhanced the mitochondrial membrane potential of aged mice, suggesting benefits for healthy ageing (Schloesser et al., 2015).
Lipoic acid	PD	Rotenone-induced PD albino rat model (≥ 22-months-old) – Treatment for 60 days (start 1h before rotenone administration and continuous daily dose until de 60 days)/ Oral gavage.	Attenuated the rotenone-induced brain toxicity, preserving the striatal ultrastructure integrity (Abdin and Sarhan, 2011).
	AD	Transgenic SAMP8 mouse model (11-months-old) – Treatment starting at 11 months of age until they were	Improved memory and reversed indices of oxidative stress in extremely old mice (Farr et al., 2012).

(*continued on next page*)

Table 1 (*continued*)

Antioxidant molecules	Disease	Animal model/ Administration route	Main outcomes
		deceased/ Subcutaneous.	
Mitoquinone (MitoQ) – Antioxidant coenzyme Q_{10} analogue conjugated with TPP^+	AD	Transgenic 3xTg-AD mouse model (2-months-old)- Treatment for 5 months/ Oral, drinking water.	Prevented the onset of cognitive deficits and decreased oxidative stress, synaptic loss, astrogliosis, and caspase activation (McManus et al., 2011).
	PD	MPTP-induced PD C57BL/6 mouse model (8-weeks-old) – Treatment for 13 days (one day before PD-like toxic stimulus, 5 days co-administered with toxic and more 7 days)/ Oral gavage.	Improved motor functions, and protected nigrostriatal axis against MPTP-induced toxicity, decreasing the TH-positive neuronal loss (Ghosh et al., 2010).
Mito-Apocynin - Apocynin conjugated with TPP^+	PD	Transgenic MitoPark mouse model - Treatment thrice weekly from age 13 to 24 weeks/ Oral gavage.	Improved motor activity and coordination and neuroprotective effects, detected at the level of mitochondrial aconitase and oxidative damage biomarkers (Langley et al., 2017).
	PD	MPTP-induced PD C57BL/6 mouse model (8- to 10-weeks-old) – Acute and sub-acute treatments designed under a preventive paradigm. Treatment starts one day before PD-like toxic stimulus followed by: i) 3 days of co-administration with the toxic stimulus (acute); ii) 5 days of co-administration with toxic stimulus and 6 additional days (sub-acute)/ Oral gavage.	Improved motor performance and nigrostriatal dopaminergic neuron survival and attenuated the inflammatory response, decreasing the expression of pro-inflammatory proteins, including iNOS (Ghosh et al., 2016).
Plastoquinone (SkQ1) – TPP^+ derivative conjugated with plastoquinone that is delivered for mitochondria	Brain ageing and AD	Senescence-accelerated OXYS rat model (1.5-months-old) – Treatment from 1.5 to 3 months of age/ Oral, chow supplementation.	Reduced the level of mtDNA deletions in the hippocampus and slowed the accelerated ageing of the brain exhibited by this animal model (Loshchenova et al., 2015).
	AD	Senescence-accelerated OXYS rat model (4-months-old and 12-months-old) – Animals with 4 months old: treatment for 7 or 14 days; Animals with 12-month-old: treatment for 6 months/ Oral, chow supplementation.	Prevented neuronal loss and synaptic damage, attenuated the spatial learning and memory impairments, and decreased the brain levels of both amyloid-β1-42 polypeptide and hyperphosphorylated tau protein (Stefanova et al., 2016).
Szeto-Schiller tetrapeptides 31 (SS-31) – a water-soluble peptide with antioxidant and mitochondriotropic properties	AD	Transgenic APP Tg2576 AD mouse model (12-months-old) – Treatment twice a week for 6 weeks/ Intraperitoneal.	Decreased amyloid-β brain levels and mitochondrial dysfunction, maintained mitochondrial dynamics and enhanced mitochondrial biogenesis and synaptic activity (Reddy et al., 2017).
	PD	MPTP-induced PD C57 black mouse model (3-months-old)	Attenuated MPTP-induced depletion of dopamine levels in the striatum brain region and protected dopaminergic neurons against MPTP damage (Yang et al., 2009).
		Treatment 30 min. before PD-like toxic stimulus administration (a triple dose in 2h interval), with reinforcement at 1 and 12 h after last MPTP dose/ Intraperitoneal.	

nanodispersion or combined with vitamin E, did not promote any detectable positive outcomes in randomized, double-blind, placebo-controlled phase III trials for PD (Table 2). The benefits of lipoic acid in animal models of neurodegenerative diseases were also not confirmed in clinical trials for AD (Table 2). It is important to stress that the cell uptake of the antioxidant molecules mentioned in Table 1 is mainly driven by passive diffusion. Their lipophilic features lead to the accumulation in the lipid component of all biomembranes without selectivity for the mitochondrial membranes. It was indicated that the great drawback of these antioxidants molecules to support a mitochondria therapy to fight neurodegeneration is related to their low availability for the mitochondria of brain cells (Moreira et al., 2010; Cardoso et al., 2017).

To overcome the above-mentioned constraints, several lipophilic cationic compounds were engineered to target mitochondria taking advantage of the mitochondrial electrical potential (negative inside) (Frantz and Wipf, 2010; Moreira et al., 2010; Jin et al., 2014; Teixeira et al., 2017). The antioxidant molecules conjugated with the mono-charged lipophilic triphenylphosphonium (TPP^+) cation (*e.g.,* MitoQ, Mito-Apocynin, Plastoquinones SkQ1) and the *Szeto-Schiller* (SS) cell-permeable synthetic tetrapeptides with a triple-positive charge at physiological pH (*e.g.,* SS31) are representative compounds of this mitochondria-targeted strategy that were tested in animal models for AD and PD. As indicated in Table 1, these studies revealed that the TPP-based antioxidants and the SS synthetic tetrapeptides promote benefits in several biochemical and physiological parameters impaired by AD- and/or PD-like degenerative processes.

Considering the Clinical Trials database of the U.S. National Library of Medicine, only MitoQ was tested in neurodegenerative diseases patients. In fact, data obtained with animal models pointing MitoQ as a promising tool to support a mitochondria-targeted therapy to treat age-related neurodegenerative diseases. However, the results obtained in

Table 2
Clinical trials performed with the antioxidant's coenzyme Q10 and lipoic acid and with the mitochondria-targeted antioxidant MitoQ to assess their therapeutic effectiveness against AD and/or PD, highlighting the main outcomes and the administration route. Data were obtained from the ClinicalTrials.gov database provided by the U.S. National Library of Medicine (www.clinicaltrials.gov).

Antioxidant molecules	Disease – Clinical trial level (Gov identifier; year)	Drug formulation/ Administration route	Main outcomes
Coenzyme Q10	PD - Phase III (NCT00740714; 2013)	Coenzyme Q10 plus vitamin E or vitamin E as placebo/ Oral, chewable wafers	Administered to patients with early PD; no therapeutic efficacy was demonstrated thereby it was not recommended for the treatment of early PD (The Parkinson Study Group QE3 Investigators, 2014).
	PD - Phase III (NCT00180037; 2005)	Coenzyme Q10 Nanodispersion or placebo/ Oral.	Well tolerated and safe but did not display symptomatic benefits in midstage PD, thereby it was not recommended for PD treatment (Storch et al., 2007).
Lipoic acid	AD - Phase I and II (NCT00090402; 2017)	Lipoic acid plus fish oil/ Oral.	Slowed both cognitive and functional decline in mild to moderately impaired AD participants. Inconclusive therapeutic benefits require additional studies (Shinto et al., 2014).
Mitoquinone (MitoQ)	PD - PROTECT phase II (NCT00329056; 2007)	MitoQ or placebo/ Oral.	Showed no difference between MitoQ and placebo on all measured parameters related to the PD progression. MitoQ did not slow down PD progression, thereby it was not recommended (Snow et al., 2010).

clinical trials for PD were disappointing (Table 2). Despite this lack of therapeutic effectiveness against PD, the oral formulations of MitoQ showed the ability to improve the vascular endothelial function in healthy older adults that has relevance to healthy aging management (Rossman et al., 2018).

The data set in Tables 1 and 2 suggest that the antioxidants, including those engineered to target the mitochondria selectively (*e.g.,* MitoQ), are unsuitable as a therapeutic tool for AD and PD, despite their promising in pre-clinical data. As highlighted in our previous work (Mendes et al., 2021), the lack of therapeutic effectiveness of these antioxidant-based therapies indicates that the antioxidant does not fulfill the purpose for which it was engineered/selected, or the interruption of the mitochondrial oxidative stress-dependent pathological cascade is not enough to promote positive outcomes on the neurodegenerative process. It is also important to stress that these therapies were not rationalized to overcome any of the known structural mitochondrial parameters affected by the neurodegenerative process but only to avoid/attenuate the oxidative damage resulting from the overproduction of ROS by dysfunctional mitochondria. Thus, we need a new vision to rationalize the fundamental assumptions required to develop an effective mitochondria-targeted therapy to fight AD and PD, which will be discussed in light of the functional 3-D architecture of the mitochondria in the next section.

5. Final remarks – outlooking a mitochondria-targeted therapy for AD and PD

Despite more than a century of active research on the mitochondrion, from its discovery as the ubiquitous intracellular structure of the eukaryotic cell, mitochondria still hold many secrets to be unraveled. As highlighted above, currently, we know that mitochondrion supports a hub of functionalities required to ensure cell survival as well as many of the physiological requirements of multicellular life. The mitochondrial-dependent biochemical pathways connected with carbon fluxes that occur in the matrix (*e.g.,* citric acid cycle) follow a similar strategy to those driven by cytosolic enzymes (*e.g.,* glycolysis), including GTP (guanosine triphosphate, ATP equivalent) synthesis by the substrate-level phosphorylation mechanism. As rationalized by Peter Mitchell, it is the dynamic 3-D architecture of mitochondria that enables a more efficient membrane energy conservation and transduction process, which can be used to supply more than 90% of the ATP required by cells (*e.g.,* as occurs in brain cells) as well as for many other purposes, such as thermogenesis and calcium homeostasis regulation. Additionally, the fineness way how the functional assembling and organization of respiratory chain complexes provided by a specific pool of lipids in the IMM uses the NADH and $FADH_2$ redox power to reduce the oxygen to water and generate an electrochemical proton gradient also leads to the release of ROS. Mitochondrial ROS play a key role in cell signaling under physiological conditions, but mitochondrial dysfunction amplifies ROS generation to pathological levels. Thus, mitochondrial dysfunction changes the redox-dependent cell signaling cascades and can lead to the oxidative damage of cellular biomolecules (oxidative stress), including the mitochondrial ones, with consequent mitochondrial dysfunction worsening. The interlink between mitochondrial dysfunction and oxidative stress under a vicious pathological cycle seems to be highly relevant in the context of neurodegeneration. However, several antioxidant-driven approaches, including those supported by engineered mitochondria-targeted molecules, have failed when tested in clinical trials for AD and PD, suggesting that oxidative stress should be a minor branch of pathological cascade underlying neurodegeneration.

Within this framework, the development of effective mitochondria-targeted therapies for AD and PD may require at least two types of challenging approaches. First, we need a set of compounds with the ability to overcome the anomalous activity of the respiratory chain complexes (mainly complex I) and to repair the abnormal mitochondrial lipidome (mainly cardiolipins), hallmarks of AD and PD mitochondrial dysfunction. These parameters are interlinked and directly related to the 3-D architecture of the mitochondria. Second, we need a competent delivery system to overcome the different physiological barriers and to supply the mitochondria of brain cells with the selected compounds. To overcome the dysfunctional complex I, we propose redox-active compounds that can oxidize NADH in the water phase and deliver the electrons to complex III of the mitochondrial redox chain, working as a membrane electron carrier. At least two types of compounds, methylene blue and elderberry anthocyanins, are reported in the literature with redox-active features to fulfil these requirements (Wen et al., 2011; Neves et al., 2019). To modulate the mitochondrial lipidome, our best suggestion is a set of lipids rich in anionic phospholipids (mainly phosphatidylglycerols, a cardiolipin precursor) with high levels of polyunsaturated fatty acids (preferentially n-3 PUFA), which can be obtained from the chloroplasts-rich tissues of plants and green algae (da Costa et al., 2015; Mendes et al., 2021). This pool of selected lipids can be combined with the above-mentioned redox-active molecules to

engineer a nanoplatform with suitable properties to overcome the different physiological barriers and target mitochondria of the brain cells. An algae lipids-anthocyanins nanoplatform was tested in cellular models, showing competence to target and modulate mitochondria functionality as well as to overcome the rotenone- or glutamate-induced toxicity (Mendes et al., 2021). However, this and all other tested mitochondria-targeted approaches were designed under the vision that emerged from Peter Mitchell's hypothesis without considering the putative functional relevance of 3-D architecture of mitochondria revealed by Electron Cryo-Tomography studies. It seems important since data obtained with tissues of patients with Leigh syndrome suggest that it is the loss of the functional 3-D architecture of the mitochondria and not the inability of monomeric forms of the FoF_1-ATP synthase to synthesize ATP that can be associated with the progressive degeneration of the central nervous system that characterizes the disease (Barca et al., 2018; Siegmund et al., 2018). Therefore, a systematic investigation to evaluate if and how the distorted/disrupted neural networks in AD and PD brains are associated with changes in the 3-D architecture of the mitochondria can shed light on the role of mitochondria on cellular networks connectivity required for multicellular life. This research should also open new therapeutic routes to address the puzzle of neurodegenerative diseases.

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