Mitochondrial electron transport chain, ROS generation and uncoupling (Review)

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Abstract. The mammalian mitochondrial electron transport chain (ETC) includes complexes I-IV, as well as the electron transporters ubiquinone and cytochrome c. There are two electron transport pathways in the ETC: Complex I/III/IV, with NADH as the substrate and complex II/III/IV, with succinic acid as the substrate. The electron flow is coupled with the generation of a proton gradient across the inner membrane and the energy accumulated in the proton gradient is used by complex V (ATP synthase) to produce ATP. The first part of this review briefly introduces the structure and function of complexes I-IV and ATP synthase, including the specific electron transfer process in each complex. Some electrons are directly transferred to O_2 to generate reactive oxygen species (ROS) in the ETC. The second part of this review discusses the sites of ROS generation in each ETC complex, including sites I_F and I_Q in complex I, site II_F in complex II and site III_{Qo} in complex III, and the physiological and pathological regulation of ROS. As signaling molecules, ROS play an important role in cell proliferation, hypoxia adaptation and cell fate determination, but excessive ROS can cause irreversible cell damage and even cell death. The occurrence and development of a number of diseases are closely related to ROS overproduction. Finally, proton leak and uncoupling proteins (UCP_S) are discussed. Proton leak consists of basal proton leak and induced proton leak. Induced proton leak is precisely regulated and induced by UCPs. A total of five UCPs (UCP1-5) have been identified in mammalian cells. UCP1 mainly plays a role in the maintenance of body temperature in a cold environment through non-shivering thermogenesis. The core role of UCP2-5 is to reduce oxidative stress under certain conditions, therefore exerting cytoprotective effects. All diseases involving oxidative stress are associated with UCPs.

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1. Introduction

The chemiosmotic theory proposed by Peter Mitchell (1) in 1961 states that the transfer of electrons derived from substrate oxidation and ATP synthesis are coupled in the mitochondrial ETC, but that does not mean that the transfer of electrons is 100% efficient. Due to the existence of electron leak and proton leak, not all electrons in the ETC can be transferred to the final electron acceptor O_2 and the energy released by the transferred electrons cannot be completely coupled with ATP generation. However, both the ROS generated by electron leak and the UCPs implicated in proton leak play an important role in the physiology and pathology of cells. Therefore, it is extremely important to understand the process of electron transfer in the ETC and the mechanism of electron leak and proton leak.

In this review, the basic components of the ETC are discussed and the process of electron transfer in each complex, including the structure, composition and function of each complex is reviewed. In addition, the ROS generation sites in the ETC are summarized and the ROS regulation is mentioned. Moreover, proton leak is emphatically introduced, including the structure, tissue distribution, functions and regulatory factors of UCPs. The diseases implicated in ROS or UCPs are simply summarized.

2. Mitochondrial ETC and ATP synthase

The ETC, which is composed of transmembrane protein complexes (I-IV) and the freely mobile electron transfer carriers ubiquinone and cytochrome c, exists in the folded inner membranes called cristae (Fig. 1). The complexes must

be assembled into a specifically configured supercomplex to function properly (2,3). These assembled components together with F_1F_0ATP synthase (namely, complex V) become the basis of ATP production during oxidative phosphorylation (OXPHOS). To better understand the whole process of how electron transportation produces ATP via the ETC, it is necessary to know the ultrastructure and function of the individual complexes.

Complex I (CI). CI, also called NADH-ubiquinone oxidoreductase, is the largest multisubunit enzyme complex in the ETC. The key role of CI is to transfer electrons from matrix NADH to ubiquinone, as the name implies. A number of studies have reported the structure of the bacterial mitochondrial CI using X-ray crystallography at a nearly atomic resolution (4,5). Mitochondria from the *Bos taurus* heart have been regarded as the best model for human CI (6-9). These studies demonstrate that the L-shaped eukaryotic CI contains two domains: The membrane arm embedded in the inner membranes and the matrix arm protruding into the matrix. The two domains are mainly composed of 14 core subunits that are conserved from bacterial CI and are the core of the enzymatic reaction. There are 45 clearly identified proteins that participate in the formation of the core subunits. The matrix arm contains seven core subunits (NDUFS1, NDUFS2, NDUFS3, NDUFS7, NDUFS8, NDUFV1 and NDUFV 2) that contain the following cofactors: A flavin mononucleotide (FMN) molecule; 7-9 FeS clusters [including the $(2Fe\text{-}2S)_{N1b}$, $(4Fe\text{-}4S)_{N3}$, $(4Fe\text{-}4S)_{N4}$, $(4Fe\text{-}4S)_{N5}$, $(4Fe\text{-}4S)_{N6a/b}$ and $(4Fe\text{-}4S)_{N2}$ clusters] (4,10); and the final electron accepting iron-sulfur cluster (N2 cluster), which was recently found to deliver electrons to ubiquinone binding sites (11). The membrane arm contains seven hydrophobic subunits (ND1-6 and ND4L), all of which are encoded by the mitochondrial genome. In addition, a large number of accessory subunits are arranged around the core subunits. The assembly of these modules has been reviewed in detail elsewhere (12). An FMN bound at the cusp of the matrix arm could form $FMNH_2$ by accepting a pair of electrons derived from matrix NADH, which is primarily produced by the tricarboxylic acid (Krebs) cycle that continuously occurs in the matrix. These interactions also mean that electrons go into the ETC and are then passed to ubiquinone via a chain of iron-sulfur clusters arranged from low to high potential [the transfer order was reported as FMN→N3→N1b→N4→N5→N6a→N6b→N2 (4)]. The ubiquinone binding site is located at the junction of the membrane arm and matrix arm, in which ubiquinone (CoQ) is reduced to ubiquinol (QH_2). Then, the conformational changes of the N2 cluster induce the formation of a proton translocation channel by the ND1, ND3, ND6 and ND4L subunits near the CoQ binding site (13). The energy released by the transfer of a pair of electrons from NADH to CoQ in CI probably (not definitively) induce the pumping of four protons from the matrix into the intermembrane space (14-17). Several hypotheses exist in current research: Ohnishi (18) proposed a hypothesis that two protons are indirectly pumped out in a conformation-coupled manner and that the other two protons are directly pumped out by the induction of ubiquinone redox. Sazanov and Hinchliffe (4) hypothesized that three protons are indirectly pumped via three antiporter homologs, and the final proton is shifted in an unclear way. In addition, Tan *et al* (14) speculated that the conformation changes and the density of water molecules in the trans-membrane domain determine the proton translocation in CI. However, how the energy transfers from the redox reaction to proton translocation are still unknown.

Complex II (CII). CII, namely, succinate dehydrogenase, is a component of the Krebs cycle as well as the ETC, serving as a link between metabolism and OXPHOS (19,20). As a part of the Krebs cycle, CII catalyzes the oxidation of succinate to fumarate. CII is another entry point for electrons and donates them from succinate to CoQ via FeS clusters, similar to CI. CII consists of four subunits (20). A total of two of the subunits, the membrane-anchor proteins CybL and CybS, are hydrophobic, anchor the complex to the inner membrane, and contain the CoQ binding site. The other two subunits are located on the matrix side of the inner membrane and contain the binding site of the substrate succinate, three FeS clusters [(2Fe-2S), (4Fe-4S) and (3Fe-4S)], and a flavoprotein covalently bound to a FAD cofactor. The assembly steps of the four subunits are detailed elsewhere (21). FAD is reduced to $FADH_2$ after receiving electrons from succinate and then transfers the electrons to FeS clusters. Then, CoQ is reduced to QH_2 after obtaining the electrons from the FeS cluster (3Fe-4S) (22). Electron transport in CII is not accompanied by the translocation of protons.

Complex III (CIII). CIII is commonly referred to as a cytochrome bc_1 complex, or CoQ-cytochrome c reductase and transfers the electrons carried by QH_2 to cytochrome c. CIII is a symmetrical dimer with 11 subunits per monomer (23). The catalytically active subunits are cytochrome b (b_L and b_H), cytochrome c_1 and a high-potential (2Fe-2S) cluster wrapped by an iron-sulphur protein (24). There are two CoQ binding sites on both ends of cytochrome b embedded in the inner membrane of the mitochondria, one of which is the QH_2 oxidation site (Q_o) located at the cytoplasmic side, which is related to the low potential cytochrome b_L. The other is the Q^- reduction site (Q_i) on the side of the matrix, which is related to the high potential cytochrome b_H (25). The electron transfer process of CIII is accomplished by the Q-cycle (24-27). QH_2 is oxidized to ubisemiquinone (QH^-) after transferring an electron to the (2Fe-2S) cluster and two protons are concurrently released into the mitochondrial intermembrane space (IMS) from the matrix (28). The (2Fe-2S) cluster transfers this electron to cytochrome c_1, from which it is transferred to cytochrome c, a mobile electron carrier. Then, the highly reductive QH^- formed at the Q_o site rapidly transfers the second electron to cytochrome b_L, which in turn transfers it to cytochrome b_H at the Q_i site. Reduced cytochrome b_H transfers this electron to the CoQ of the Q_i site, forming a QH^-. To complete the Q-cycle, the second QH_2 molecule is oxidized at the Q_o site while displacing the other two protons. Similarly, one electron is transferred to the (2Fe-2S) cluster and the other electron to cytochrome b_H and finally to QH^- of the Qi site to produce QH_2.

Complex IV (CIV). CIV, also known as cytochrome c oxidase, transfers electrons from cytochrome c to the terminal electron acceptor O_2 to generate H_2O. Mammalian CIV consists of 13

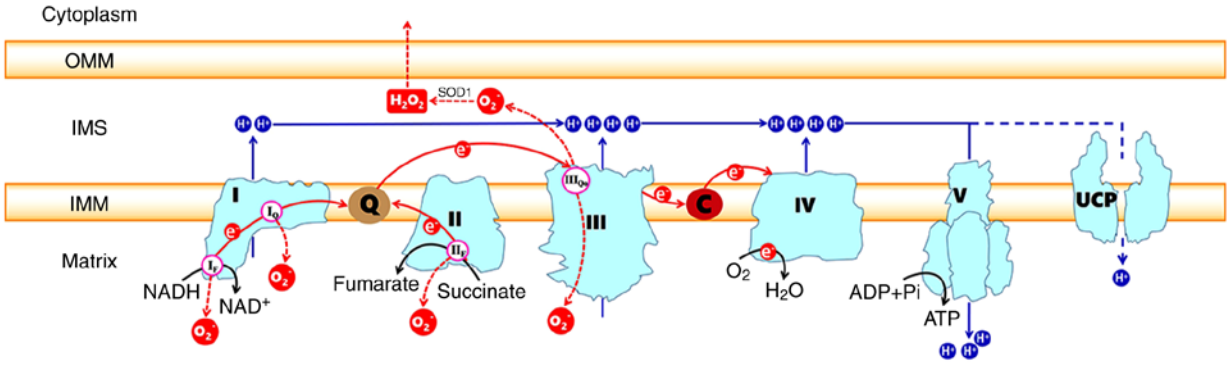

Figure 1. Generation of electron leaks and proton leaks in the electron transport chain. Electrons derived from oxidizable substrates are passed through CI/III/IV or CII/III/IV in an exergonic process that drives the proton pumping into the IMS of CI, CIII and CIV. The energy of the proton gradient drives the ATP synthesis of CV or can be consumed by UCPs. The sites of superoxide production in each complex are also indicated, including sites I_F and I_Q in CI, sites II_F in CII and site III_{Qo} in CIII. The O_2^- released into the IMS by site III_{Qo} can be converted into H_2O_2 in a reaction catalyzed by superoxide dismutase 1 and H_2O_2 then may diffuse into the cytoplasm. The red arrows indicate electron pathways. The black arrows represent substrate reactions. The blue arrows show the proton circuit across the IMM. In cyan, the complexes I-V are marked as I, II, III, IV, V, respectively. Q, ubiquinone; C, cytochrome c; IMM, inner mitochondrial membrane; IMS, intermembrane space; OMM, outer mitochondrial membrane; UCP, uncoupling protein.

different subunits containing four redox-active metal centers, namely, Cu_A, heme a (Fe_a) and a binuclear center composed of heme a_3 (Fe_{a3}) and Cu_B (29,30). Subunits I, II, III are encoded by mitochondrial DNA and are the core subunits, while the 10 nuclear-coded subunits are the accessory subunits (31,32). Subunit I contains three of the four cofactors, heme a and the binuclear center, which transfers electrons from heme a to O_2 (29). Subunits II and III are located on both sides of subunit I and there are two Cu_A cofactors on the side of the intermembrane space of subunit II. Subunit III stabilizes the other two core proteins and is mainly involved in proton pumping (33,34). The nuclear-coded subunits participate in the modulation of physiological activity via the allosteric ATP-mediated inhibition of CIV, which depends on the ATP/ADP-ratio (35-39).

Cytochrome c, similar to CoQ, is a mobile electron carrier that is loosely connected to the outer surface of the inner mitochondrial membrane by electrostatic interactions, allowing it to interact with the cytochrome c_1 of CIII and to accept electrons (39). The reduced cytochrome c moves along the surface of the membrane and interacts with subunit II of CIV by electrostatic interactions, simultaneously transmitting electrons to the Cu_A site of subunit II, and then the electrons are passed from heme a to the binuclear center of subunit I (29,39), where the O_2 is reduced to H_2O. A total of four electrons at a time from cytochrome c are almost simultaneously transferred to bind dioxygen; eight protons in total are removed from the matrix, of which half are used to form the two water molecules and the other four are pumped across the membrane into the IMS (40).

Complex V (CV). CV is normally called F_1F_0 ATP synthase and consists of two functional domains: F_0 and F_1. The F_0 domain, located in the inner mitochondrial membrane, contains a subunit c-ring, including one of each of the subunits a, b, d, F6 and oligomycin sensitivity-conferring protein (OSCP) as well as the accessory subunits e, f, g and A6L (41,42). The subunits b, d, F6 and OSCP form the peripheral stalk, which is located on one side of the complex. A number of additional subunits (e, f, g and A6L), which all span the membrane, are associated with the c-ring subunit. The F_1 domain, situated in the mitochondrial matrix, consists of soluble subunits: Three α subunits, three β subunits and one of each of the γ, δ and ε subunits (42,43). The three α and three β subunits make up the catalytic head of F_1, and the γ, δ and ε subunits constitute the central stalk that connects the F_1 head and F_0 subunit c-ring (41,43,44). The ETC transfers two electrons at a time to monooxygen to generate one H_2O molecule, which is accompanied by the pumping of four, four and two protons from the matrix to the IMS through CI, CIII, and CIV, respectively (or zero, four and two protons through the CII, CIII, and CIV, respectively). Then, the protons pass from the IMS to the matrix through F_0, which transfers the stored energy created by the proton electrochemical gradient to F_1, causing a conformational change in F_1F_0 ATP synthase so that ADP can be phosphorylated to form ATP (41).

In conclusion, the entire composition of each individual complex has been well described over the past century and it is now widely accepted that these complexes must establish interactions and form supercomplexes to perform their function. Due to the application of cryo-electron microscopy, a greater understanding of the high-resolution structure of these complexes has been gained (45-47).

3. ROS generation in the ETC

The sites of ROS production in the ETC. Mitochondria are a main source of cellular ROS. Under physiological conditions, 0.2-2% of the electrons in the ETC do not follow the normal transfer order but instead directly leak out of the ETC and interact with oxygen to produce superoxide or hydrogen peroxide (48,49). A total of 11 sites that produce superoxide (O_2^-) and/or hydrogen peroxide (H_2O_2) that are associated with substrate oxidation and the ETC have currently been identified in mammalian mitochondria (50). Sites O_F, P_F, B_F and A_F are in the 2-oxoacid dehydrogenase complexes, sites I_F and I_Q are in CI, site III_{Qo} is in CIII, and sites II_F, G_Q, E_F and D_Q are linked to the Q-dependent dehydrogenases in the QH_2/Q pool (50). The occurrence of numerous diseases and hypoxia are closely related to the increase of ROS production. CI and

CIII, especially CI, are considered to be the main sites of ROS production in mitochondria (51,52).

ROS can be generated in the matrix at both site I_F (FMN site) and site I_Q (CoQ binding site) during the transfer of electrons from NADH to CoQ in CI (Fig. 1). Rotenone and piericidin are site I_Q inhibitors that interrupt the electron transfer to CoQ and increase ROS production at site I_F. Hernansanz-Agustin *et al* (53) found that acute hypoxia produces a superoxide burst during the first few minutes in arterial endothelial cells and CI mainly participated in this process.

CII produces ROS at site II_F (Fig. 1), which is associated with succinate dehydrogenase. The level of ROS produced by site II_F under normal conditions is negligible, but the increases in ROS observed in CII mutation-related diseases are mainly derived from site II_F (54,55). The study of isolated mitochondria from rat skeletal muscle also indicated that the maximum capacity for ROS production of site II_F is very high, exceeded only by site III_{Qo} and perhaps site I_Q (50,56). The capacity of site II_F to produce ROS is closely related to the quantity of reduced flavoprotein, whose FAD is a potent site of electron leakage to generate ROS. ROS are exclusively produced in the matrix, because the flavoprotein is located on the matrix side of the inner mitochondrial membrane (56). In addition, any contribution by site II_F can be dampened by the occupation of the CII flavoprotein site by dicarboxylic acids, particularly oxaloacetate, malate and succinate, which blocks the access of oxygen to site II_F, where it would form ROS (21,57).

CIII produces small amounts of ROS, which could be overlooked compared to the ROS production of CI (52). CIII transfers electrons through the Q-cycle. In this process, ubisemiquinone (QH^-) of the Q_o site carrying a single electron can move freely in CIII, directly leaking the single electron to O_2, forming ROS through a nonenzymatic reaction (58,59). The formed ROS can be released into both the matrix and IMS despite the location of the Q_o site on the IMS side of the inner mitochondrial membrane. Muller *et al* (60) built two models explaining how superoxide can reach the matrix. The O_2^- released into the IMS can be converted to the relatively more stable form of H_2O_2 by superoxide dismutase (SOD) enzymes (Fig. 1). This permanent and stable oxidant molecule, which freely disperses through the outer membrane of mitochondria, acts as an intracellular signaling molecule, physiologically functioning via the direct modification of amino acids (61). However, supporting evidence demonstrates that O_2^- can permeate through the mitochondrial membrane into the cytosol through anion channels (62). Treberg *et al* (63) experiments in the mitochondria of wild-type rat skeletal muscle proved that ~63% of ROS are produced in the matrix. Antimycin A can specifically block the Q_i site of CIII, resulting in the stalling of electrons on the QH^- at site III_{Qo}, which could react with O_2 to generate ROS (64,65). As specific inhibitors of the Q_o site, stigmatellin and myxothiazol can block the binding of QH_2 to the Q_o site, which also blocks the transfer of electrons into CIII, thereby preventing the production of ROS in CIII (64). Previously, a chemical suppressor of site III_{Qo} electron leak called S3QELs was screened and found to specifically suppress the ROS formation at site III_{Qo} without affecting electron transport or the redox states of other centers (66).

CIV is less prone to produce ROS when O_2 is bound to Fe_{a3}^{2+} or when O_2 is negatively polarized (O_2^-) and expected to undergo a structural change. This structural change allows O_2^- to receive three electron equivalents from Cu_B^{1+}, Fe_{a3}^{3+} and the hydroxyl group of Tyr244 (Tyr-OH) in no particular order, providing the complete reduction of O_2 and minimizing the production of ROS (67). It is important to note that the binuclear center structure of CIV is crucial for the nonsequential transfer of the three electron equivalents (39,67).

ROS as signaling molecules in physiological or pathological conditions. In the past, it was believed that ROS were exclusively harmful to cells. However, recent studies have demonstrated that ROS appear to be very important second messengers that mediate different intracellular pathways (50,61,68). ROS act through the oxidative modification of numerous types of proteins, particularly receptors, kinases, phosphatases, caspases, ion channels and transcription factors (68). The ROS produced from CIII are necessary for HIF-1α stabilization and consequently, for the proliferation of cells, including vascular smooth muscle cells, endothelial cells and erythroid progenitors (69). There is ample evidence that ROS are also involved in different protein kinase signaling cascades, such as the protein kinase B (AKT), AMP-activate protein kinase (AMPK) and mitogen-activated protein kinase kinase kinase/mitogen-activated protein kinase 8 pathways, changing the fate of cells between autophagy and apoptosis [(Table I and (70)]. Under hypoxic conditions, ROS activate AMPK, which can upregulate cytoprotective autophagy by inhibiting downstream mammalian target of rapamycin activity (71). ROS have also been demonstrated to regulate synaptic plasticity-related signalling molecules, receptors and channels, including N-methyl-d-aspartate receptor (72), Ca^{2+} channel (73,74), Ca^{2+} kinase II (CaMKII) (75), extracellular signal-regulated kinase (76) and cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) (74,77). ROS are also necessary for long-term potentiation, a phenomenon of synaptic plasticity widely regarded as one of the main molecular mechanisms that form the basis of learning and memory (77,78). Physiological levels of ROS can promote the establishment of neuronal polarity and regulate neuronal cytoskeletal organization and dynamics by regulating intracellular Ca^{2+} release (79-81).

The amount of ROS generated as a result of a stimulus determines whether ROS play beneficial or harmful roles, which means different physiological or pathological pathways are activated. A large amount of ROS cause lipid peroxidation, DNA damage, protein oxidation, irreversible impairment of mitochondria, insufficient ATP generation and, eventually, cell death (82). The ROS-mediated activation of NHE-1 is implicated in cardiac hypertrophy (83). In addition, the activation of CaMKII by ROS contributes to an increase in cardiomyocyte death and the development of heart failure (84). ROS are involved in a number of chronic inflammatory diseases, particularly atherogenesis, through activating the NF-κB pathway (85). In addition, it is widely known that the ROS burst during reperfusion plays a critical role in ischemia-reperfusion

Table I. The signaling pathways involved in the different cell fates in which the mitochondrial production of ROS has been implicated.

Cell fates	Signaling pathways	(Refs.)
Apoptosis	i) Death receptor pathway: ROS-Death receptors (FasL, TNF-α, TRAIL)-Caspase-8-Caspase-3 ii) Mitochondrial pathway: ROS-Apoptosome complex (cytochrome c, Apaf-1 and dATP)-Caspase-9-Caspase-3 iii) ROS-ASK1-JNK-Death receptor pathway/Mitochondrial pathway	(87-91)
Autophagy	i) ROS-FOXO3-LC3/BNIP3-Autophagy ii) ROS-NRF2-p62-Autophagy iii) ROS-HIF-1-BNIP3/NIX-Autophagy iv) ROS-TIGAR-Autophagy v) ROS-Atg4-LC3-II-Autophagy vi) ROS-AMPK-ULK1 complex-Autophagy	(92-97)
Necrosis	i) ROS-death receptors (TNF-α)-RIPK1-Necrosome (RIPK3 and MLKL) ii) ROS-PARP1-Necrosome (RIPK3 and MLKL) iii) ROS-p53-Bax-MPTP opening-Necrosis	(98-101)
Pyroptosis	i) ROS-MAPK-ERK1/2-NLRP3 inflammasome ii) ROS-TXNIP-NLRP3 inflammasome	(102-104)

ROS, reactive oxygen species; ERK, extracellular signal regulated kinase; MAPK, mitogen associate protein kinase; HIF, hypoxia inducible factor; AMPK, AMP-activated protein kinase; TNF, tumor necrosis factor; MPTP, mitochondrial permeability transition pore; NLRP3, NACHT, LRR and PYD domains-containing protein 3; RIPK1, receptor-interacting serine/threonine-protein kinase 3; MLKL, mixed lineage kinase domain-like protein.

Table II. The pathologies in which the mitochondrial production of reactive oxygen species has been implicated.

Pathology	Representative references
Atherosclerosis	(105,106)
Hypertension	(107)
Ischemia-reperfusion injury	(86,108,109)
Cardiomyopathy	(110,111)
Pulmonary hypertension	(112,113)
COPD	(114-116)
Cancer	(117,118)
Diabetes	(119,120)
Non-alcoholic liver disease	(121,122)
Alzheimer's disease	(119,123)
Parkinson's disease	(124)
Schizophrenia	(125,126)
Hearing loss	(127,128)
Age-related macular degeneration	(129,130)
Obesity	(131)
HIV-1 infection	(132,133)
Duchenne muscular dystrophy	(134,135)
Periodontitis	(136)

COPD, chronic pulmonary obstructive disorder; HIV, human immunodeficiency virus.

injury (86). Table II summarizes the pathologies in which ROS has been implicated.

4. Mitochondrial proton leak

The overview of proton leak. OXPHOS is not completely coupled. Under routine circumstances, a small number of protons do not pass through ATP synthase and instead flow directly into the mitochondrial matrix across the inner mitochondrial membrane, without the generation of ATP, in a process known as proton leak. In the concept of 'respiratory state' proposed by Chance and Williams (16), mitochondrial respiration persists in the absence of ADP (state 4) and reflects the oxygen consumption of proton leak. The existence of proton leak can also be proven by the collapse of the proton gradient (Δp) in the presence of the ATP synthase inhibitor oligomycin in isolated mitochondria (137).

It was found that the proton leak of the inner mitochondrial membrane demonstrated nonohmic conductivity (137). According to Ohm's Law (R=U/I), the resistance of a conductor is fixed and the electric current increases linearly with increasing voltage. However, the rate of proton leak increases exponentially with increasing ΔΨ. That is, the proton conductivity increases when ΔΨ is high. The existence of nonohmic conductivity indicates that there is a bidirectional self-regulatory mechanism between electron transport and proton re-entry: Protons are pumped out of the matrix into the IMS driven by the electron transport in CI, CIII, CIV, and ΔΨ is gradually elevated. On one hand, the elevated ΔΨ inhibits the transfer of electrons to further elevate ΔΨ, through which the inner membrane is protected from electric shock and maintains suitable ΔΨ. On the other hand, the exorbitant ΔΨ can cause the increase in proton leak to decrease.

Proton leak consists of two parts: Basal proton leak and inducible proton leak. Basal proton leakage is not regulated and is related to the lipid bilayer of the inner mitochondrial membrane and the adenine nucleotide translocase (ANT). Induced proton leak is precisely regulated and can be catalyzed or suppressed by uncoupling proteins (UCPs) and ANT.

Basal proton leak has an important relationship with the basal metabolic rate (BMR) in mammals at rest. The lower the BMR of a species, the weaker the basal proton conductance. Studies have demonstrated that the extent of basal proton leak among species has a phylogenetic relationship (138,139). Although the lipid bilayer can significantly increase proton conductivity, only ~5% of basal proton leak is mediated by lipid bilayers (140) and most of the basic proton leak is correlated with ANT (141). UCP1, which is abundant in brown adipose tissue (BAT), may also be involved in basal proton leak (142), although there remains controversy (143). In particular, proton leak through ANT or UCP1 is independent of protein activity, as proton leak still occurs in the presence of the ANT inhibitor carboxyatractylate and the UCP1 inhibitor GDP (141,144).

The majority of the induced proton leak is catalyzed by UCPs. UCPs belong to the family of mitochondrial anion carrier proteins, through which the protons can reflux into the matrix. To date, five UCPs have been identified in mammals, UCP1, UCP2, UCP3, UCP4 and UCP5, and all are present in the form of dimers in the inner mitochondrial membrane (145). These UCPs have a purine nucleotide binding site located on a projection in the IMS (146). The purine nucleotides (ATP, ADP, GTP and GDP) are inhibitors of UCP-mediated proton flux, whereas ROS and fatty acids are activators (147,148). In addition to the role of uncoupling, UCPs may also participate in other processes, such as the regulation of calcium homeostasis, ion transportation or synaptic plasticity (149,150).

UCP1-5. UCP1 is mainly expressed in BAT, which converts stored energy in Δp into heat for thermogenesis (151). UCP1 can also be detected in the beige adipocytes of white adipose tissue (WAT) during thermal acclimation under specific conditions (152). The genetic deletion of UCP1 severely inhibits cold adaptive thermogenesis and diet-induced adrenergic thermogenesis, and UCP1-null mice develop fatal hypothermia upon cold exposure (153,154). Interestingly, WAT can exert nonshivering thermogenesis with a UCP1-independent pathway (155,156). UCP1 has also been found in thymocytes and demonstrated to be involved in the maturation and fate determination of developing T-cells (157-159). Sale *et al* (160) found that UCP1 is expressed in islets and associates with the acute insulin response to glucose.

UCP1-catalyzed proton leak could be activated by long chain free fatty acids and inhibited by purine nucleotides (161). Acute cold or overfeeding stimuli induce the release of norepinephrine by sympathetic nerves and then induce cAMP-responsive pathways through β3-adrenergic receptors on brown adipocytes, which could further promote the transcription of UCP1 and lipolysis for more free fatty acids (162). There are currently three models for the regulated mechanism of UCP1-implicated proton leak (162-169).

UCP2 and UCP3, paralogues of UCP1, exhibit ~60% sequence identity with UCP1 and ~70% identity with each other (170,171). UCP2 is rather ubiquitous, expressed in WAT, BAT, macrophages, erythroid cells, thymocytes and pancreatic β-cells as well as heart, brain, lung, kidney and lymphocytes (172-176). The UCP3 gene is mainly expressed in skeletal muscle, BAT and heart (177,178) and has also been detected in the thymus, spleen (179) and skin cells (180). Studies with UCP2/3-ablated mice have demonstrated that UCP2 and UCP3 are not implicated in adaptive thermogenesis or the regulation of body weight (170,181). However, the role of UCP2 and UCP3 in inhibiting the production of ROS in mitochondria by reducing $\Delta\Psi$ is widely accepted (182). A strong correlation between ROS production and mitochondrial membrane potential ($\Delta\Psi$) has already been confirmed (183). Experiments have demonstrated that ROS production is increased in UCP2/3-ablated mice (184-186). ROS-induced lipid hydroperoxides such as hydroxynonenal can activate UCP2/3-mediated proton leak, but the mechanism remains uncertain (178). By reducing $\Delta\Psi$, the transfer of electrons in the ETC can be accelerated and the likelihood of electrons being directly transferred to O_2 can be minimized. Therefore, mild uncoupling is a feedback mechanism adopted by the body to prevent excessive ROS in the mitochondria, which was termed 'uncoupling to survive' (187). However, whether UCP1 is implicated in the regulation of ROS in BAT is still controversial (175,188,189). In addition to the function of reducing the generation of ROS, UCP3 has been demonstrated to be involved in exporting mitochondrial fatty acid anions to the cytoplasm, thereby protecting the mitochondrial against lipid peroxide-induced damage (190,191).

UCP4 and UCP5 (also called brain mitochondrial carrier protein 1), which have 30% homology to UCP1 (192), are primarily expressed in the central nervous system of mammals (193,194). Although UCP4 and UCP5 are more widely distributed in the brain than UCP2, less is known about their function. UCP4 was first detected in the brain, but it has recently been found in adipocytes (195). In addition, UCP4 also plays a predominant role in insect mitochondria (196). On the other hand, UCP5, which is not limited to the brain, is also expressed in the testis, uterus, kidney, lung, stomach, liver and heart (149). It has been demonstrated that neuronal UCP4 and UCP5 share similar conformational and proton transport activities with UCP1-UCP3 (149,197). Although UCP4 and UCP5 may play an unconfirmed role in the neural system, their function for reducing oxidative stress is clear (195,198). Hoang *et al* (149) speculated that UCP4 acts in a neuroprotective role during early neuronal development, while UCP2 and UCP5 provided the protective function of restricting ROS production in developed neurons and other tissues, based on the observation that UCP2 and UCP5 displayed higher proton transport rates than UCP4. Oxidative stress has been proven to be involved in both neurodegenerative diseases and aging, so the UCP-dependent reduction of ROS in the nervous system has the potential to be neuroprotective in diseases such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (199,200). Certain evidence indicates that protein-protein interactions exist between UCP4 and CII: UCP4-overexpressing neuroblastoma cells increase ATP synthesis via increasing the succinate-induced respiration mediated by CII (201). Pfeiffer *et al* (202) demonstrated that the *Caenorhabditis elegans* UCP4 plays a novel role in the regulation of CII by controlling succinate transport into

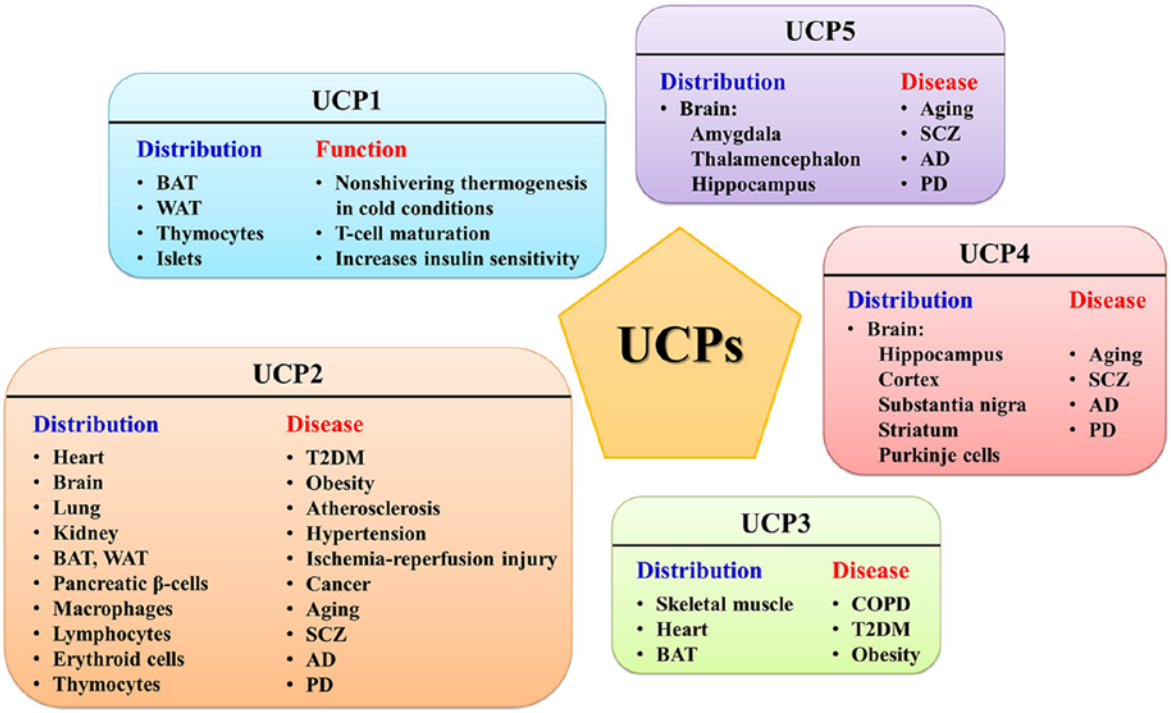

Figure 2. Distribution of mitochondrial UCP1-5 and their related diseases. BAT, brown adipose tissue; WAT, white adipose tissue; T2DM, type II diabetes; SCZ, schizophrenia; AD, Alzheimer's disease; PD, Parkinson's disease; COPD, chronic obstructive pulmonary disease; UCP, uncoupling proteins.

mitochondria. UCP4 was also deemed to regulate calcium homeostasis in neuronal cells (203).

UCPs and diseases. Regardless, as an inner mitochondrial membrane protein, UCP1 mainly plays a role in the maintenance of body temperature in a cold environment through non-shivering thermogenesis and UCP2-5 can protect cells from oxidative stress by reducing the mitochondrial membrane potential via mediating uncoupling. Due to their wide distribution, UCPs have different physiological significance in specific tissues. Therefore, abnormal changes in UCPs in each tissue will lead to tissue-specific diseases (Fig. 2).

The ubiquitous UCP2 is associated with a number of metabolic diseases, such as diabetes, obesity, cardiovascular disease and even cancer, which has created immense interest in exploring the relationship between UCP2 and these diseases. Since UCP2 can regulate fatty acid and lipid metabolism, a number of studies have confirmed that UCP2 overexpression is associated with diet-induced obesity (204-206). UCP2 is highly expressed in pancreatic β-cells and has a negative regulatory effect on insulin secretion. Robson-Doucette *et al* (207) in 2011 demonstrated that the overexpression of UCP2 could reduce the secretion of glucose-induced insulin and subsequently induce type II diabetes (T2DM). Moreover, UCP2 knockout mice exhibited hyperinsulinemia and hypoglycaemia (208). Briefly, glucose is metabolized through the ETC to increase ATP production, which leads to the release of insulin and the production of ROS. Chronic elevated glucose status can lead to the excessive expression of UCP2 to reduce the overproduction of ROS, resulting in reduced ATP production, reduced insulin secretion and, eventually, progression to diabetes (209,210). Chronic inflammation, including atherosclerosis, hypertension, diabetic vasculopathy and ischemia-reperfusion injury, is accompanied by excessive ROS production, which means that UCP2 can play a protective role in these diseases by reducing oxidative stress. The signaling pathways, such as nuclear factor (NF)-κB and p53, that can lead to cellular senescence, inflammation, and irreversible vasoconstriction can be inhibited by reducing ROS production. A study showed that the protein levels of UCP2 were significantly higher in human tumor tissues from the head and neck, skin, prostate and pancreas (211). Although the role of UCP2 in tumors is intuitive, the regulatory effects of UCP2 on cellular glucose and lipid metabolism, as well as the regulation of cellular oxidative stress, may be related to the development of tumors. Several studies have confirmed that UCP2 overexpression provides protection for tumor cells and leads to chemoresistance (212-214).

UCP3 has been verified to be associated with exercise intolerance in chronic obstructive pulmonary disease (COPD) patients. COPD patients have exhibited impairment in the form of exercise intolerance, which was linked to increased levels of intramuscular lipid peroxidation products (114,215). Given the fiber-type-specific expression of UCP3, researchers have examined UCP3 levels in muscle biopsies from COPD patients and found that UCP3 content was reduced in COPD (216). It can be speculated that low muscle UCP3 levels contribute to impaired exercise tolerance in COPD patients based on the function of fatty acid anion transportation. In addition, a number of studies have demonstrated that the accumulation of lipid peroxide damage, resulting from decreased UCP3 in skeletal muscle, leads to excessive oxidative stress and is a crucial aspect in the development and progression of obesity and T2DM (191,217,218).

UCP4, UCP5, together with UCP2, are expressed in the nervous system and are implicated in several neurological

disorders, such as schizophrenia (SCZ), Alzheimer's disease (AD), and Parkinson's disease (PD). Various studies link UCP2 with neurodegeneration and aging (123,124,219). The three UCPs exert neuroprotective effects mainly through the alleviation of oxidative stress. The results from selected single nucleotide polymorphism markers within the neuronal UCPs showed that UCP2 and UCP4 are important in the genetic etiology of SCZ (219). Surprisingly, despite the downregulation of UCP2 mRNA levels in SCZ patients, Gigante *et al* (220) found that there were no differences in UCP2 protein between patients and controls. Future studies will be necessary to clarify whether the mechanism of UCP2 is protective and opposes SCZ progression. Furthermore, in the brains of AD patients, the expression levels of UCP2, 4, and 5 were significantly reduced, which limited the activation of cytoprotective mechanisms to slow the progress of AD (124). UCP5 and, especially, UCP4 are linked to PD. UCP4 is regulated by the oxidized DJ-1 and partially via the NF-κB pathway and can protect against oxidative stress and stabilize Ca^{2+} homeostasis in PD, as demonstrated by Ramsden *et al* (148) in 2012. Drugs that induce neuronal UCP expression might represent another effective strategy to ameliorate PD.

5. Concluding remarks and perspective

In conclusion, the ETC is the core component of mitochondria. The OXPHOS process in the ETC, coupled with the generation of ATP, also results in ROS production. As a double-edged sword, ROS can play a role in signaling pathways, but ROS overproduction can cause cellular damage. The ROS produced by CIII is not only released into the matrix but also released into the IMS. The ROS released into the IMS can be converted to H_2O_2 in a reaction catalyzed by SOD1, and the H_2O_2 may diffuse out of the mitochondria and play an important role in physiological and pathological pathways. Therefore, the artificial regulation of ROS at the CIII site (site III_{Qo}) may be of great significance. Although the precise mechanism of ROS production is still unclear, the use of specific ROS inhibitors to reduce the excessive production of ROS under pathological conditions has ameliorated oxidative stress-mediated diseases. UCP-mediated proton leak is a positive feedback mechanism for the protection of cells against oxidative stress due to the rapid production of ATP. The proper activation of UCPs can reduce the production of cellular ROS without causing a decrease in ATP; however, when the expression of UCPs becomes too high or too low or the UCP genes are mutated, pathological effects that are involved in various diseases can occur. UCP1 mediates the inducible proton conductance that is responsible for non-shivering thermogenesis in BAT, a critical response to prolonged cold exposure. UCP2 is involved in a variety of diseases, such as diabetes, obesity and cardiovascular disease. In addition, UCP2, UCP4 and UCP5 play an important role in neuroprotection and are associated with neurological diseases such as SCZ, AD and PD. Drugs targeting UCP expression and activity might represent as an effective strategy to ameliorate these diseases. However, the detailed mechanisms of the role of UCPs and the regulation of UCP expression under normal and stressful situations warrant further exploration.

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Authors' contributions

ZBY and LZ conceived the review and analyzed the relevant literature. RZZ and SJ collected and reviewed the literature related to the topic of this manuscript and drafted the main part of this manuscript. RZZ, LZ and ZBY critically revised the manuscript. RZZ and SJ produced the figures. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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