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Review Article

Structure and mechanism of mitochondrial electron transport chain

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ABSTRACT

Respiration is one of the most vital and basic features of living organisms. In mammals, respiration is accomplished by respiratory chain complexes located on the mitochondrial inner membrane. In the past century, scientists put tremendous efforts in understanding these complexes, but failed to solve the high resolution structure until recently. In 2016, three research groups reported the structure of respiratory chain supercomplex from different species, and fortunately the structure solved by our group has the highest resolution. In this review, we will compare the recently solved structures of respirasome, probe into the relationship between cristae shape and respiratory chain organization, and discuss the highly disputed issues afterwards. Besides, our group reported the first high resolution structure of respirasome and medium resolution structure of megacomplex from cultured human cells this year. Definitely, these supercomplex structures will provide precious information for conquering the mitochondrial malfunction diseases.

Mitochondria are involved in a variety of vital cellular activities, among which energy conversion is the most critical. A vast amount of efforts have been put into depicting the structure, assembly, coupling mechanism and pathology of respiratory chain complexes, and several landmarks should be noticed: 1) Mitchell in 1961 put forward the chemiosmotic hypothesis [1], which is supported by later structural and functional analyses, especially the structure of bovine ATP synthase solved in 1994 by Walker [2]; 2) Schägger in 2000 solubilized the mitochondria membrane with digitonin and detected the high molecular weight bands using blue native page (BNPAGE), thus for the first time proposing the respirasome model [3], which is further amended by Enriquez who verified the respiratory activity of respirasome and raised the plasticity model in 2008 [4]; 3) Our group presented the first near-atomic resolution structure of respirasome in 2016 [5], together with several nominal resolution structures independently solved by Sazanov and Kuhlbrandt almost at the same time [6,7], again heating up debates about the electron transfer mechanism in electron transport chain supercomplex. As exemplified above, our understanding

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of the energy conversion machinery has been greatly advanced, however, this understanding is still far from satisfying.

The plasticity model is thus far the most widely accepted theory about the respiratory chain organization [8,9]. In this model, most CII, CIV and a relevant proportion of CIII stand alone and seem to move freely on the inner mitochondrial membrane, while the majority of CI is stabilized by the CIII dimer, with or without several copies of CIV, and the super-assembly of these complexes are dynamic, with a certain turnover rate. Consequently, electron transfer from CI to CIII is most likely carried out by CoQs shuttled within the $I_1III_2IV_n$ supercomplexes, despite that CoQs carrying electrons from CII seems to freely move on the membrane. Kinetic evidence using flux control analysis performed by Lenaz and Genova is consistent with this model, showing that electron transfer via CI and CIII behaves like an integral enzyme, while the transfer via CII and CIII demonstrates like separate enzymes [10,11].

However, the exact organization of the respiratory chain has never gained consensus and has been challenged continuously by emerging evidence. It has been shown that the stoichiometry of supercomplexes can vary in different physiological conditions and cell types [12–14], but we know nearly nothing about the regulation pathway. Most recently, Greggio reported that after 4 months of exercise training, not only individual respiratory chain complexes but also respiratory supercomplexes in human muscle mitochondria show an increase, and the free complexes tend to assemble into functional supercomplexes after exercise [15]. But still, we don't understand the detailed signaling pathways. Cryo-ET (Cryo-electron tomography) analyses locate the ATP synthase dimers at the edge of the cristae curve, and CI at the approximately plane surface of cristae [16,17]. The shape of mitochondria cristae is closely related with aging and cell apoptosis, and has been reported to influence the supercomplex assembly [18,19]. However, the detailed connection between cristae shape and supercopmlex organization is not known, either. Structural evidence indicate the existence of higher oligomerization level of respirasomes, termed as megacomplexes [5,6,9], and finally in this year, our group unprecedentedly reported the first medium resolution structure of megacomplex from cultured human cells after docking of the well resolved sub regions including CI, CIII dimer and CIV [20]. For the first time, this work provided solid evidence for the existence of megacomplex, observed the possible compartment of Q pool, and connected the organization of respiratory chain more tightly with the shape of cristae. Since this is the first structure of human respirasome, previously reported mitochondrial disease related mutations can all be mapped into our structure. Doubtlessly, this is a great step forward into conquering many severe neurodegenerative diseases, including Alzheimer's syndrome, Parkinson's disease, multiple sclerosis, friedreich's ataxia, Amyotrophic lateral sclerosis, etc. [20].

Disputes about substrate channeling, electron transfer mechanism and the assembly process of supercomplexes have always been fierce [21]. Despite the widely accepted segmentation theory of the Q pool, Hirst et al. presented evidence against the partitioning of the Q pool in 2014 through their spectroscopic and kinetic experiments [22]. In accordance, since the early 1980s, Gutman et al. have shown the phenomenon of reverse electron transfer from succinate to NAD+, indicating that the Q pool in SC might have communications with the free Q pool via some unknown mechanism [23]; in contrast to the classic Q cycle theory, our group proposed another electron pathway within CIII based on our high resolution structure of respirasome [9]; In addition, although CIV subunit COX7A2L in mouse has been identified as an assembly factor of supercomplex, more assembly factors still await to be found and the accurate assembly process is far from clear [24].

In this review, we will introduce recent advances of the mitochondrial ETC (electron transport complexes) research in three parts: the structure details of respirasome, the relationship between cristae shape and respiratory chain organization, and the highly disputed issues including substrate channeling, electron transfer pathway, and the assembly process of respirasome.

Structures of respirasome

Since the August of 2016, structural research of respiratory chain has obtained exciting breakthroughs, including four medium to high resolution cryo-EM structures of respirasome independently solved by three different groups [5–7,9]. These structures originate from porcine, bovine or ovine, and due to differences in species source, purification methods and cryo-EM data quality, some detailed discrepancies were observed while the overall structures fit well with each other [Fig. 1].

Most recently (Oct 5th, 2017), a review article from Sazanov's group also discussed current knowledge about mitochondrial respiratory chain, but clearly they have some misunderstanding about our work [25]. They criticized that the interaction sites between CI and CIII are not resolved at the level of side chains, which is not true. In fact, our density map has the highest resolution by far, and the contact sites can be identified clearly and shown in the Figure 5F of our published paper [5]. They also pointed out that the density for CIV is weak so the positioning and inferred contacts may not be reliable, which is quite arbitrary. In fact, after docking the crystal CIV structure (PDB:1OCC) into our map, the correlation coefficients reach a very high level, which means the final map is quite reliable. Besides, they accused that the maps describing the intermediate states of processing are of the wrong hand, which is actually not important and indicates that maybe the authors are not quite familiar with the structural solving process of the single particle cryo-EM method, because in the final map we performed a total reversion and the opposite chirality of the intermediate states is only for convenience in data processing. Finally, they claimed that the 10-kDa subunit (NDUFV3) is misassigned, which is not true because in samples from porcine heart the density for this subunit is missing, and the reason is the difference in species source. Inexplicably, the authors cited one of our piezo1 structural paper [26] (cited as the 74th citation) in the "Function of supercomplex formation" section when they described the "Comparison of the structures of isolated CI and SCs" [25]. It's really unintelligible to cite one of our paper there. As stated above, despite some defects caused by the limitation of current technology, we still get the best density map till now.

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