

This is the final peer-reviewed accepted manuscript of:

The mitochondrial energy conversion involves cytochrome c diffusion into the respiratory supercomplexes.

Nesci S, Lenaz G. Biochim Biophys Acta Bioenerg. 2021; 22:148394.

The final published version is available online at: <https://doi-org.ezproxy.unibo.it/10.1016/j.bbabi.2021.148394>

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

The mitochondrial energy conversion involves cytochrome *c* diffusion into the respiratory supercomplexes

Salvatore Nesci¹, Giorgio Lenaz²

¹Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Via Tolara di Sopra, 50, 40064, Ozzano Emilia (BO), Italy; ²Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum University of Bologna, Via Zamboni, 33, 40126, Bologna (BO), Italy.

Correspondence: salvatore.nesci@unibo.it (S.N.); giorgio.lenaz@unibo.it (G.L.)

Mitochondrial respiration is sustained by a series of enzyme complexes, namely NADH-ubiquinone oxidoreductase (complex I, CI), ubiquinol-cytochrome *c* oxidoreductase (complex III, CIII; also known as the cytochrome *bc*₁ complex) and cytochrome *c* oxidase (complex IV, CIV); this electron transport chain (ETC) delivers the reduced equivalents from substrates to oxygen driven by redox potential. The electron flow coupled to H⁺ pump activity of complex I, III, and IV generates the transmembrane electrochemical gradient of H⁺ across the inner mitochondrial membrane (IMM). The ETC is composed by electron carriers placed into respiratory complexes and two mobile carriers: coenzyme Q (Q) is hydrophobic and lipid-soluble, whereas cytochrome *c* (cyt. *c*) is hydrophilic and anchored on the positive side of the IMM. The segregation of both Q and cyt. *c* into two distinct sub-domains depend on the organization of the respiratory chain in free or supercomplexes (SCs) organization. The mitochondrial architecture of respiratory complexes has been identified into the “respirasome”, an arrangement of supercomplex C₁C_{III}₂C_{IV} [1]; its formation is important for the stability of the ETC complexes and for reducing the production of reactive oxygen species, while a possible kinetic advantage through substrate channelling is still debated [2,3].

Structural and kinetic evidence suggests that Q channelling occurs between CI and CIII in the respirasome. The observations in favour of channelling are the following: (a) the sites for Q in either CI or CIII are situated at a distance ensuring fast micro-diffusion of Q between them; (b) in flux control analysis of NADH oxidation the high flux control coefficients of both CI and CIII indicate that they behave as a single enzyme; (c) many studies have highlighted a rate advantage in the SC vs dissociated complexes; (d) the path of Q diffusion between CI and CIII appears to be separated from that between CII or other FAD-dehydrogenases and CIII (cf [3]). Criticisms to the suggestion of Q channelling have been advanced in some papers from Hirst’s laboratory, indicating a single homogeneous Q pool [2]; the main findings are (a) absence of additivity in the simultaneous aerobic oxidation of NADH and succinate, arguing against the existence of two separate fluxes, and (b) rapid oxidation of NADH by heterotopic alternative oxidase, that receives electrons from Q, arguing against an obligatory path from CI to CIII via bound Q. We are convinced, however, that the contrasting results are due to confounding experimental conditions: in (a) the fluxes are mixed at the level of cyt. *c* (see below), whereas in (b) inhibitors downhill the Q region (CIV) force the dissociation of bound Q from the SC (cf [4]), obliging it to assume pool behaviour.

The matter seems to be different for the interaction of cyt. *c* between CIII and CIV. In the metabolic flux control analysis CIV has a low flux control coefficient in presence of CI substrate, thus indicating that CIV is not rate-limiting step and arguing against channelling [5]. In mammalian mitochondria, as used in the flux control study of Bianchi et al [5], the majority of CIV and cyt. *c* are free: most electron transfer would occur via diffusion of the cyt. *c* pool and channelling within the SC, if occurring, would be obscured. On the contrary, in plant mitochondria, where cyt. *c* is more tightly bound, the flux control coefficients of CIV are coherently high indicating channelling (cf. [3]).

In yeast mitochondria, at difference with mammalian mitochondria, there is no CI and all CIV is bound to CIII in a SC III₂IV_{1/2} [6]; using such system, Berndtsson and colleagues [7] reported that the respiratory control ratios (state 3/state 4), which is a measure for the coupling between substrate oxidation and phosphorylation, is unaltered in *wild type* and mutated CIII-subunit Cor1 where the CIII₂-CIV interaction is disrupted. The explanation is a decrease of the respiration rate in both State 3 and State 4. Indeed, the electron transfer from CIII to CIV via cyt. *c* is inefficient in the absence of SC formation, but the overexpression of cyt. *c* or *in vitro* addition of exogenous cyt. *c* restore the efficiency of cellular energy conversion [7]. Cyt. *c* as a mobile electron carrier ensures electron transfer of respiration in a diffusion-dependent manner between CIII and CIV. The enzymatic kinetics of redox reaction of cyt. *c* channelling within the respirasome may explain the “boost” of respiration when the SC is present [8]. Therefore, in disassembled respirasome CIII can reduce only the cyt. *c* pool while in the SC the redox cycling of cyt. *c* is sustained by the shortened distance for its diffusion, as cyt. *c* can reside either in a tighter binding in the SC or in a free pool in the aqueous phase (Figure 1). Theoretical realistic assumptions indicate that electron transfer between complexes III and IV can become rate limiting and that in turn it is affected by the equilibration time of cyt. *c* in the volume of the intracristae space. Hence, there is a kinetic advantage of bringing complexes III and IV together in the membrane to form supercomplexes [9]. Therefore, in disassembled respirasome the CIV can reduce only the cyt. *c* pool while in the SC the redox cycling of cyt. *c* is sustained by the shortened distance for its diffusion. This different nature of cyt. *c* could justify the improvement of mitochondrial energy conversion in respiratory SCs. Indeed, cyt. *c* binding properties, also depending on post-translational changes, appear to be the key for controlling its mechanism of electron transfer by “surfing” between CIII and CIV in response to cellular bioenergetic demands [10].

The molecular interaction between *bound-free* cyt. *c* (Figure 1) and respirasome is determined by stoichiometric ratio of soluble electron carrier and binding sites. The SC structural data indicate a surface-associated cyt. *c* movement where the negatively-charged surface region of the CIII₂CIV allows an electron transfer of the positively charged cyt. *c* by 2D diffusion [11]. Indeed, SCs structure improve the electron transfer through the ETC during respiration by means of the structural arrangement of CIII and CIV that creates a cyt. *c* binding sites of both the respiratory complexes in the immediate vicinity [1]. Therefore, diffusion-limited electron transfer reactions might be different from electron transfer reactions between two kinetically linked complexes because electron exchange by cyt. *c* between CIII and CIV is more efficient in the respirasome than between the separated complexes. The cyt. *c* electrons delivered from CIII to CIV decrease upon disruption of respiratory SCs, but this deficiency in disassembled respirasome can improve by increasing the levels of cyt. *c* [7].

In mitochondrial respiration the reduction of cyt. *c* is catalyzed by asymmetric function of CIII₂ monomers [12] and the dimeric form of CIII has simultaneously active only one monomer during normal turnover of electron and substrate transfer. The spatial organization of the active CIII monomer faces among the Q and cyt. *c* sites on CI and CIV, respectively providing support for direct electron transfer in mitochondrial respiration with substrate channeling in the CI CIII₂CIV. Indeed, the active CIII monomers is spatially placed in a linear arrangement with CI and CIV in the respirasome and the site-by-site distance between respiratory complexes disadvantages the diffusion in the lipid bilayer of the mobile carrier which encounters the active CIII monomer first.

The proximity between CIII and CIV, with no direct protein-protein contacts but interactions, prevents conformational changes required during the cyt. *c* transfer, where the position of CIV relies on the structural state of the respirasome identified as “tight” and “loose” [1]. However, the structures of SCs present open active sites to the membrane of CIII and CIV without barriers to diffusion of cyt. *c*_(free) in the intermembrane space. Nevertheless, this does not exclude the possibility of concomitant substrate channeling where the respiration is more rapid and efficient by cyt. *c* exchange between CIII and CIV in presence of SC.

On balance, the physiological function of mitochondrial respiratory SCs remains elusive, but respirasomes enhance electron transfer flow in particular by CIV incorporation into SCs through Subunit Complex Assembly

Factor 1 (SCAF1), resulting in improvement of the metabolic fitness by mitochondria adaptation to cell energy demands [13].

Why appreciable channeling may not exist at the level of cyt. *c* in mammalian mitochondria? Mammalian mitochondria have several dehydrogenases directing electrons to Q, however they usually have only one oxidase (CIV) receiving electrons from CIII via cyt. *c*. For this reason, whilst it may be useful to separate the major NADH-dependent flux from CI from those departing from succinate, fatty acid oxidation and other metabolic pathways by separating the Q compartments [14], there is no such need for cyt. *c* that is by and large receiving electrons univocally from CIII. On the contrary, plant mitochondria have complex branched pathways in the cyt. *c* region [15] and therefore they may need separation and control of different fluxes. Therefore, the cyt. *c* is bound either to CIII or CIV or at intermediate position in SC with the role of supporting the QH₂-O₂ oxidoreductase activity of CIII₂CIV [11]. The functional and structural evidence on the cyt. *c* shuttling electrons along the respiratory chain corroborate the link with formation or dissociation of SC in the regulation of mitochondrial respiration.

References

- [1] J.A. Letts, K. Fiedorczuk, L.A. Sazanov, The architecture of respiratory supercomplexes, *Nature*. 537 (2016) 644–648. <https://doi.org/10.1038/nature19774>.
- [2] J. Hirst, Open questions: respiratory chain supercomplexes-why are they there and what do they do?, *BMC Biol*. 16 (2018) 111. <https://doi.org/10.1186/s12915-018-0577-5>.
- [3] G. Lenaz, G. Tioli, A.I. Falasca, M.L. Genova, Complex i function in mitochondrial supercomplexes, *Biochimica et Biophysica Acta - Bioenergetics*. 1857 (2016) 991–1000. <https://doi.org/10.1016/j.bbabi.2016.01.013>.
- [4] M. Szibor, T. Gainutdinov, E. Fernandez-Vizarrá, E. Dufour, Z. Gizatullina, G. Debska-Vielhaber, J. Heidler, I. Wittig, C. Viscomi, F. Gellerich, A.L. Moore, Bioenergetic consequences from xenotopic expression of a tunicate AOX in mouse mitochondria: Switch from RET and ROS to FET, *Biochim Biophys Acta Bioenerg*. 1861 (2020) 148137. <https://doi.org/10.1016/j.bbabi.2019.148137>.
- [5] C. Bianchi, M.L. Genova, G. Parenti Castelli, G. Lenaz, The mitochondrial respiratory chain is partially organized in a supercomplex assembly: kinetic evidence using flux control analysis, *J Biol Chem*. 279 (2004) 36562–36569. <https://doi.org/10.1074/jbc.M405135200>.
- [6] J. Heinemeyer, H.-P. Braun, E.J. Boekema, R. Kouril, A structural model of the cytochrome C reductase/oxidase supercomplex from yeast mitochondria, *J Biol Chem*. 282 (2007) 12240–12248. <https://doi.org/10.1074/jbc.M610545200>.
- [7] J. Berndtsson, A. Aufschnaiter, S. Rathore, L. Marin-Buera, H. Dawitz, J. Diessl, V. Kohler, A. Barrientos, S. Büttner, F. Fontanesi, M. Ott, Respiratory supercomplexes enhance electron transport by decreasing cytochrome *c* diffusion distance, *EMBO Rep*. (2020) e51015. <https://doi.org/10.15252/embr.202051015>.
- [8] F. den Brave, T. Becker, Supercomplex formation boosts respiration, *EMBO Rep*. (2020) e51830. <https://doi.org/10.15252/embr.202051830>.
- [9] A. Stuchebrukhov, J. Schäfer, J. Berg, P. Brzezinski, Kinetic advantage of forming respiratory supercomplexes, *Biochim Biophys Acta Bioenerg*. 1861 (2020) 148193. <https://doi.org/10.1016/j.bbabi.2020.148193>.
- [10] G. Pérez-Mejías, A. Guerra-Castellano, A. Díaz-Quintana, M.A. De la Rosa, I. Díaz-Moreno, Cytochrome *c*: Surfing Off of the Mitochondrial Membrane on the Tops of Complexes III and IV, *Comput Struct Biotechnol J*. 17 (2019) 654–660. <https://doi.org/10.1016/j.csbj.2019.05.002>.
- [11] A. Moe, J.D. Trani, J.L. Rubinstein, P. Brzezinski, Cryo-EM structure and kinetics reveal electron transfer by 2D diffusion of cytochrome *c* in the yeast III-IV respiratory supercomplex, *BioRxiv*. (2020) 2020.11.27.401935. <https://doi.org/10.1101/2020.11.27.401935>.
- [12] J.S. Sousa, D.J. Mills, J. Vonck, W. Kühlbrandt, Functional asymmetry and electron flow in the bovine respirasome, *Elife*. 5 (2016). <https://doi.org/10.7554/eLife.21290>.

- [13] C. García-Poyatos, S. Cogliati, E. Calvo, P. Hernansanz-Agustín, S. Lagarrigue, R. Magni, M. Botos, X. Langa, F. Amati, J. Vázquez, N. Mercader, J.A. Enríquez, Scaf1 promotes respiratory supercomplexes and metabolic efficiency in zebrafish, *EMBO Rep.* 21 (2020) e50287. <https://doi.org/10.15252/embr.202050287>.
- [14] A. Guarás, E. Perales-Clemente, E. Calvo, R. Acín-Pérez, M. Loureiro-Lopez, C. Pujol, I. Martínez-Carrascoso, E. Nuñez, F. García-Marqués, M.A. Rodríguez-Hernández, A. Cortés, F. Díaz, A. Pérez-Martos, C.T. Moraes, P. Fernández-Silva, A. Trifunovic, P. Navas, J. Vazquez, J.A. Enríquez, The CoQH2/CoQ Ratio Serves as a Sensor of Respiratory Chain Efficiency, *Cell Rep.* 15 (2016) 197–209. <https://doi.org/10.1016/j.celrep.2016.03.009>.
- [15] P. Schertl, H.-P. Braun, Respiratory electron transfer pathways in plant mitochondria, *Front Plant Sci.* 5 (2014) 163. <https://doi.org/10.3389/fpls.2014.00163>.

Figure caption

Figure 1. Supercomplex CIII₂CIV redox reaction of free and bound cyt. *c*. Electron-transfer bridge from CIII to CIV via a tightly bound cyt. *c* or electron transfer between CIII and CIV occurs via the cyt. *c* pool. Dimer of complex III (CIII₂), complex IV (CIV), cardiolipin (CL), cyt. *c* tightly bound in the CIII₂CIV (cyt. *c*_(bound)), electrons transfer with the cyt. *c* pool (cyt. *c*_(free)). The enzymes are drawn as ribbon representations obtained from modified PDB ID code: 2YBB.

