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

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

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Mitochondrial respiration is sustained by a series of enzyme complexes, namely NADH-ubiquinone oxidoreductase (complex I, *C*I), ubiquinol-cytochrome *c* oxidoreductase (complex III, *C*III; also known as the cytochrome bc_1 complex) and cytochrome *c* oxidase (complex IV, *C*IV); 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 *C*I*C*III$_2$*C*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 *C*I and *C*III in the respirasome. The observations in favour of channelling are the following: (a) the sites for Q in either *C*I or *C*III 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 *C*I and *C*III 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 *C*I and *C*III appears to be separated from that between *C*II or other FAD-dehydrogenases and *C*III (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 *C*I to *C*III 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 (*C*IV) 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 *C*III and *C*IV. In the metabolic flux control analysis *C*IV has a low flux control coefficient in presence of *C*I substrate, thus indicating that *C*IV 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 *C*IV 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, were cyt. *c* is more tightly bound, the flux control coefficients of *C*IV 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 $III_2IV_{1/2}$ [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 *C*III-subunit Cor1 where the *C*III_2–*C*IV interaction is disrupted. The explanation is a decrease of the respiration rate in both State 3 and State 4. Indeed, the electron transfer from *C*III to *C*IV 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 *C*III and *C*IV. 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 *C*III 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 *C*IV 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 *C*III and *C*IV 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 *C*III_2*C*IV 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 *C*III and *C*IV 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 *C*III and *C*IV is more efficient in the respirasome than between the separated complexes. The cyt. *c* electrons delivered from *C*III to *C*IV 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 *C*III_2 monomers [12] and the dimeric form of *C*III has simultaneously active only one monomer during normal turnover of electron and substrate transfer. The spatial organization of the active *C*III monomer faces among the Q and cyt. *c* sites on *C*I and *C*IV, respectively providing support for direct electron transfer in mitochondrial respiration with substrate channeling in the *C*I*C*III_2*C*IV. Indeed, the active *C*III monomers is spatially placed in a linear arrangement with *C*I and *C*IV 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 *C*III monomer first.

The proximity between *C*III and *C*IV, with no direct protein-protein contacts but interactions, prevents conformational changes required during the cyt. *c* transfer, where the position of *C*IV 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 *C*III and *C*IV 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 *C*III and *C*IV in presence of SC.

On balance, the physiological function of mitochondrial respiratory SCs remains elusive, but respirasomes enhance electron transfer flow in particular by *C*IV 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 (*C*IV) receiving electrons from *C*III via cyt. *c*. For this reason, whilst it may be useful to separate the major NADH-dependent flux from *C*I 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 *C*III. 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 *C*III or *C*IV or at intermediate position in SC with the role of supporting the QH_2-O_2 oxidoreductase activity of *C*III$_2$*C*IV [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.

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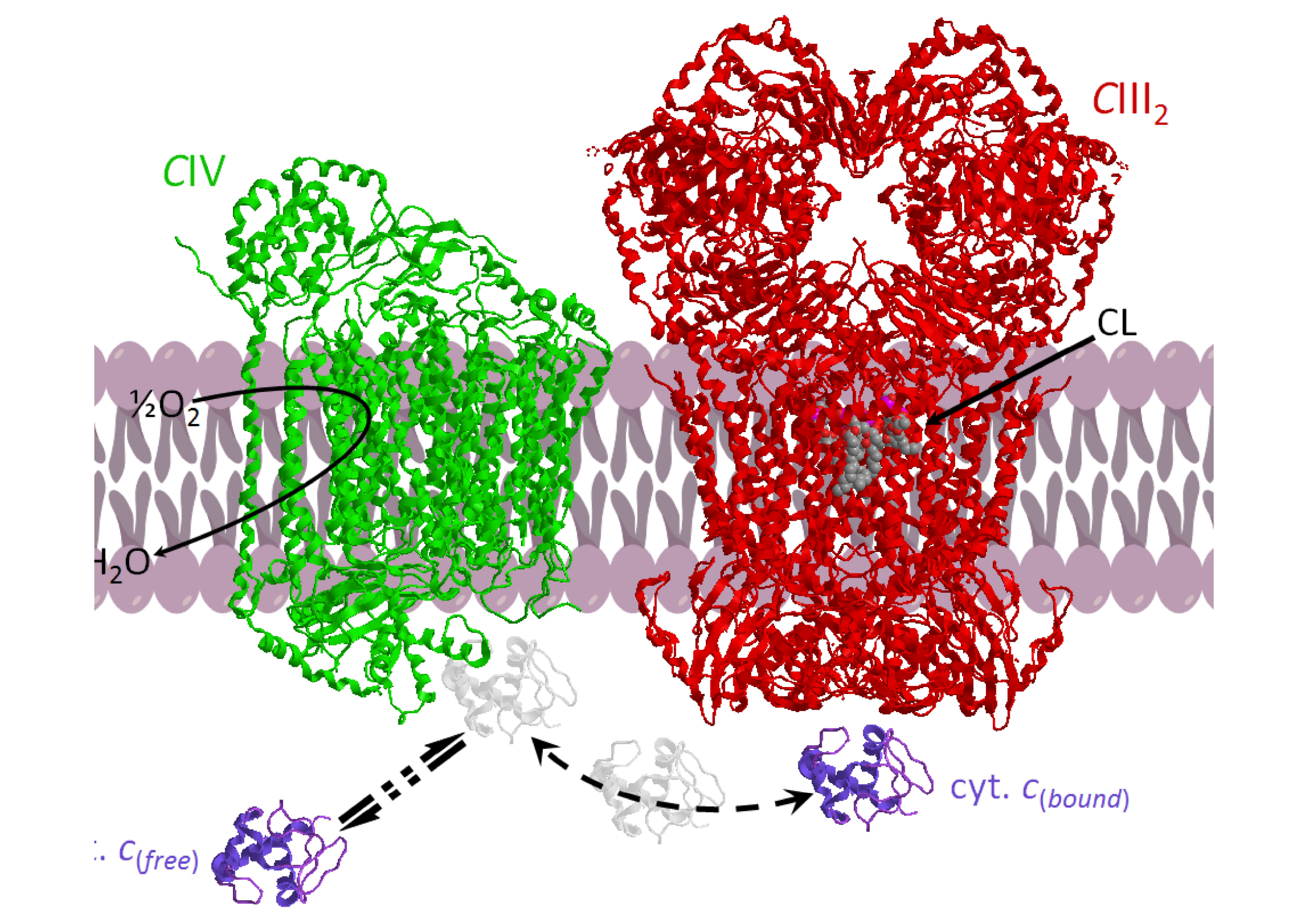
Figure caption

Figure 1. Supercomplex *C*III$_2$*C*IV redox reaction of free and bound cyt. *c*. Electron-transfer bridge from *C*III to *C*IV via a tightly bound cyt. *c* or electron transfer between *C*III and *C*IV occurs via the cyt. *c* pool. Dimer of complex III (*C*III$_2$), complex IV (*C*IV), cardiolipin (CL), cyt. *c* tightly bound in the *C*III$_2$*C*IV (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.


CIII2
CIV
CL
½O2
H2O
cyt. c(bound)
c(free)