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I Function, Therefore I Am: Overcoming Skepticism about Mitochondrial Supercomplexes

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ABSTRACT

The mitochondrial respiratory chain is believed to dynamically arrange in suprastructures known as supercomplexes or respirasomes, though their function remains elusive. A recent study in Science (Lapuente-Brun et al., 2013) now *reports that dynamic supercomplex assembly determines electron flux from different substrates through the respiratory chain.*

The structural and functional organization of the mitochondrial respiratory chain (MRC) has been a matter of intense debate for nearly 60 years. Accumulated evidence has demonstrated that in a variety of organisms, MRC complexes I, III and IV (CI, CIII, CIV) associate to form different supramolecular assemblies known as supercomplexes (SC) or respirasomes (Chance and Williams, 1955; Schagger and Pfeiffer, 2000; Cruciat et al., 2000). Although the functional significance of mitochondrial supercomplexes has been historically questioned, recent evidence supports that: (i) Some supercomplex species respire (Acin-Perez et al., 2008) and may integrate the “mobile” electron donors coenzyme Q (CoQ) and cytochrome *c* (cytc) to confer kinetic advantage by facilitating efficient electron transfer through partner redox components (substrate channeling) (Genova and Lenaz, 2013); (ii) MRC supercomplex assembly minimizes the generation of reactive oxygen species from CI (Maranzana et al., 2013), and (iii) Mammalian CI is assembled and stabilized in supercomplex associations (Moreno-Lastres et al., 2012) (**Figure 1**).

The MRC arrangement into individual enzymes or diverse supercomplexes was postulated to be a dynamic process already 30 years ago (Hochman et al., 1982; Acin-Perez et al., 2008). In a recent issue of Science, an attractive role for this dynamic supercomplex assembly as a mechanism to determine electron flux from different substrates through the MRC has been reported (Lapuente-Brun et al., 2013). By combining blue native analyses with measurements of MRC enzyme activities and oxygen consumption in mouse cells partially depleted of complexes I and III, the authors showed that electron

flux from CI to CIII proceeds essentially within supercomplexes, whereas electron flow from complex II (CII) preferentially occurs through CI-unbound complex III. The dynamic association/dissociation of the MRC complexes would thus define how competition among substrates is set to gain simultaneous access to the MRC. Within the MRC, the CoQ pool collects electrons from different dehydrogenases and regulates electron flow according to its redox state and amount. CII is activated by reduced quinones, while the dehydrogenase activities feeding electrons to CI rapidly slow down with the reduction state of the ubiquinone pool (Genova and Lenaz, 2013). The presence of CoQ in supercomplexes containing CI and CIII implies efficient substrate channeling within these structures, thus favoring the best environment for NADH oxidation.

A major contribution by Lapuente-Brun et al. is the identification of a novel supercomplex assembly factor, COX7A2L/COX7RP/SCAFI. SCAFI was identified in a screen for proteins present in supercomplexes but not in free complexes, and proposed to mediate interactions between CIII and CIV. The authors discovered that, whereas several mouse strains, such as CD1, NZB, 129 or CBA, express wild-type SCAFI and their mitochondria display CIV-containing supercomplexes (SC+ phenotype), including respirasomes (associations of CI, CIII and CIV), two commonly used laboratory mouse strains, C57BL/6J and Balb/cJ, lack both SCAFI and these supercomplexes (SC- phenotype). Intriguingly, CI and CII-driven respiratory rates and ATP production were higher in SC- than in SC+ liver mitochondria, suggesting that CIV bound to supercomplexes is not fully available to receive electrons from both NADH and

$FADH_2$-linked substrates. SCAFI is thus proposed to define distinct CIV populations that, according to their assembly status (free CIV or associated in supercomplexes), would allow minimizing competitive inhibition of respiration between CI and CII-driven substrates. This model assumes though that CIV-containing supercomplexes display functional cytochrome *c*, a controversial issue yet to be convincingly demonstrated (Genova and Lenaz, 2013).

The authors also tested the respiratory capacity through CI and CII of liver mitochondria from SC+ and SC- mice strains fed *ad libitum* or fasted for 18 hours to activate fatty acid degradation, since the ratio of NADH/$FADH_2$ electrons feeding the MRC is higher when glucose is the respiratory substrate and lower for fatty-acid oxidation. Their results consistently supported the existence of separate electron routes and the idea that the rearrangement of MRC super assemblies may facilitate efficient oxidation of the available substrates.

Mechanistically, however, the proposed regulation of electron flux does not necessarily imply the existence of two distinct CoQ pools, since freely diffusing CoQ species could transiently associate with supercomplexes depending on substrate availability for oxidation. Direct kinetic evidences for changes in electron flux through the MRC upon substrate variations, as well as for the CoQ exchange between the two putative pools, remain to be demonstrated, besides how this exchange is regulated upon variations in CoQ levels or redox state. The structural/functional MRC adaptations probably vary among tissues given their different metabolic rates, mitochondrial mass and tissue-specific MRC subunit isoforms. Future work should therefore explore how adaptations occur in diverse

tissues and how they are modulated by oxygen levels and oxidative stress, or during aging and disease.

The precise function of SCAFI remains unknown. Opposing to Lapuente-Brun et al., a simultaneous report showed that, in *Cox7rp*KO mice, SCAFI/COX7RP mediates respirasomes assembly to gain full activity of the MRC in skeletal muscle (Ikeda et al., 2013). Of note, *Cox7rp*KO mice were generated in the supposedly SCAFI- C57BL/6 strain, a key point to be reconciled. If SCAFI binds exclusively to supercomplexes, it should do so when CIII and CIV are already associated, supporting its role as a supercomplex glue or stabilizer. In this regard, Lapuente-Brun et al. did not discuss how the dynamic MRC rearrangement physically occurs. The current model proposes that supercomplex assembly proceeds by the incorporation of individual subunits or modules into a partially-assembled CI scaffold, rather than by the association of previously assembled enzymes. Perhaps respirasome assembly initially proceeds as proposed (Moreno-Lastres et al., 2012), and subsequently SCAFI aids the dynamic exchange of CIV, reflecting its variable stoichiometry within supercomplexes. Preassembled modules could concurrently accumulate and be ready to ignite MRC supercomplexes remodeling when required, a possibility compatible with the authors´ observations (Lapuente-Brun et al., 2013).

Yet controversial, the lack of respirasomes in some laboratory “wild-type” mice strains does not lead to evident pathological phenotypes, strongly suggesting that the respirasomes may not be the preferential functional forms of the MRC. Consistently, metabolic flux control analysis proposes the arrangement

of CI and CIII in a functional supercomplex, in which CoQ would be in dissociation equilibrium between supercomplexes-bound and free diffusing, whereas CIV would mainly act in its free form (Genova and Lenaz, 2013). This raises the interesting possibility that different supercomplexes could cope with diverse physiological functions, some probably yet to be identified, thus supporting the requirement of the proposed dynamic rearrangement.

The latest advances in the field are therefore shading light into the dynamic reorganization of the MRC enzymes into supercomplexes/respirasomes, their functional roles, biogenetic pathways and contributing factors. Despite the multiple questions remaining, it is time to overcome skepticism about the reality of mitochondrial supercomplexes.

LEGEND TO FIGURE 1.

Figure 1. Dynamic rearrangements of respiratory chain complexes and functional implications. The supramolecular organization of the mitochondrial respiratory chain (MRC) has been proposed to confer kinetic advantages on electron transfer through substrate channeling, to prevent ROS production, and to aid the assembly and stabilization of complex I (yellow banner). Lapuente-Brun and colleagues have recently reported a new functional role for the dynamic association/dissociation of MRC complexes and supercomplexes, which defines dedicated CoQ and cyt *c* pools in order to organize electron flux to optimize the use of available substrates through the respiratory chain (labeled in red) (Lapuente-Brun et al., 2013). These dynamic rearrangements range from all-bound to all-free MRC complexes, and open the possibility that different modes of MRC organization are switched on-switched off to regulate diverse physiological functions through, i.e., ROS signaling or turnover of MRC enzymes. $SCI+III_2+IV_{1-4}$ refers to the respirasomes or supercomplexes formed by the association of one complex I (CI), a complex III dimer ($CIII_2$) and one to four copies of complex IV (CIV_{1-4}). Intermediate supercomplex species can be found in nature, in combination with free complex II (CII), complex III dimers and complex IV in different stoichiometries (CIV_{1-2}). Complex I requires to be associated in supercomplexes to minimize destabilization and ROS generation.

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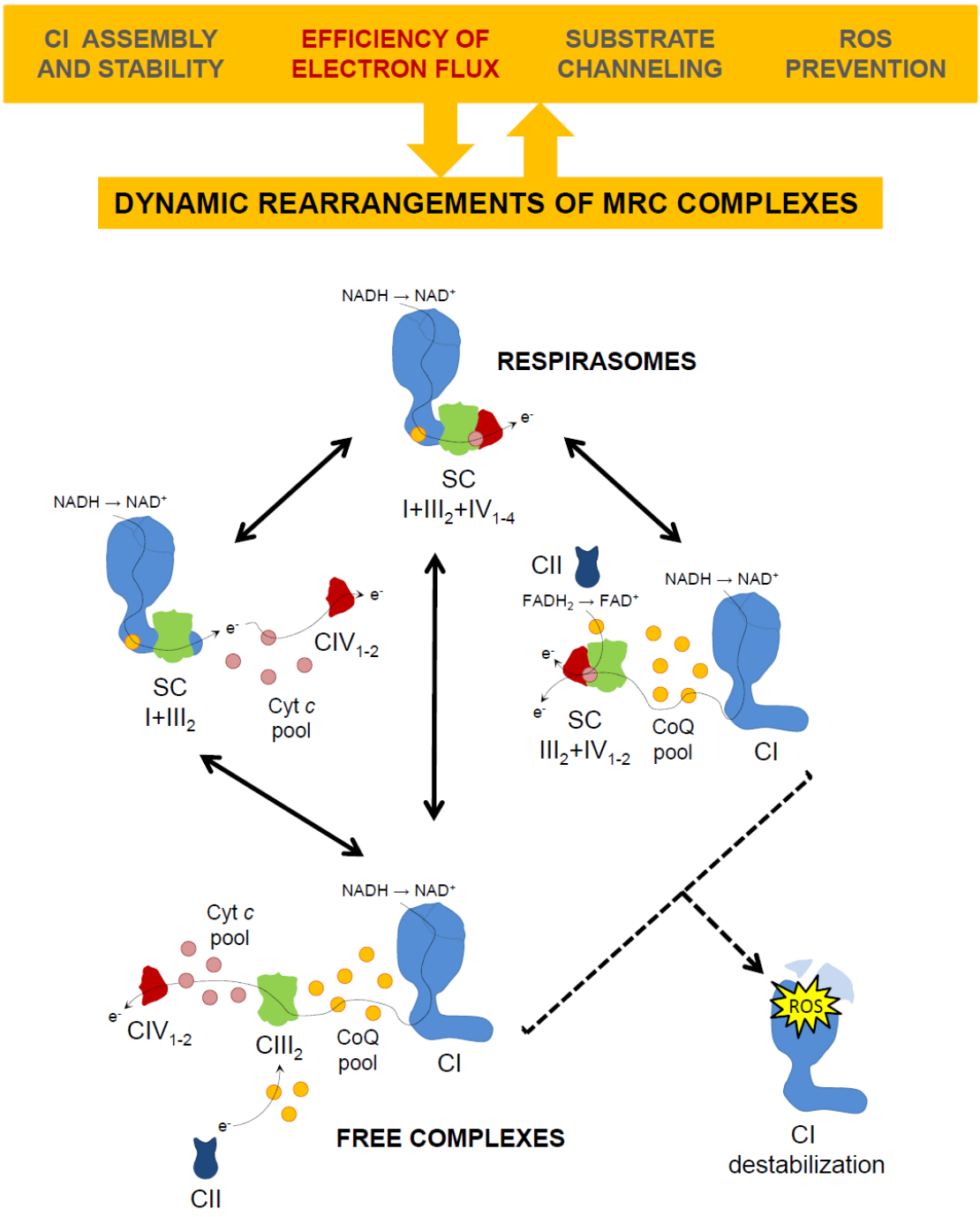
CI ASSEMBLY AND STABILITY
EFFICIENCY OF ELECTRON FLUX
SUBSTRATE CHANNELING
ROS PREVENTION
DYNAMIC REARRANGEMENTS OF MRC COMPLEXES
NADH → NAD+
RESPIRASOMES
e-
SC
I+III2+IV1-4
NADH → NAD+
e-
e-
CIV1-2
SC
I+III2
Cyt c
pool
CII
FADH2 → FAD+
NADH → NAD+
e-
e-
SC
III2+IV1-2
CoQ
pool
CI
NADH → NAD+
Cyt c
pool
e-
CIV1-2
CIII2
CoQ
pool
CI
e-
CII
FREE COMPLEXES
ROS
CI
destabilization

Figure 1