Supersizing the Mitochondrial Respiratory Chain

Eric A. Shoubridge[1,*]
[1]Department of Human Genetics, McGill University/MNI, Montreal, QC H3A 2B4, Canada
*Correspondence: eric@ericpc.mni.mcgill.ca
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The complexes of the mitochondrial respiratory chain assemble into higher-order structures called supercomplexes or respirasomes that are thought to be important in channeling electron flow and controlling ROS production. A number of recent papers identify the first protein factors necessary for supercomplex assembly and stability.

The oxidative phosphorylation system, which comprises the four multimeric enzyme complexes (CI–CIV) of the respiratory chain and the ATP synthase complex (CV), drives the synthesis of ATP in most cells. Two mobile carriers, ubiquinone and cytochrome *c*, are used to transfer electrons from NADH, produced by the oxidation of carbohydrates and lipids, to molecular oxygen (Figure 1). This creates a proton electrochemical gradient that is harnessed by CV to synthesize ATP. Although it was initially proposed that the process of electron transfer occurred by random collisions between the individual enzyme complexes, many biochemical studies suggested the existence of higher-order structures, or supercomplexes, consisting of two or more different respiratory chain complexes that could channel electron flow. With the advent of native electrophoretic gel systems that could separate large membrane complexes (Schägger and von Jagow, 1991), supercomplexes were shown in a wide variety of eukaryotes, including yeast, plants, and animals. They have been variously hypothesized to be important in increasing the efficiency of electron transport, controlling the production of reactive oxygen species (a byproduct of oxidative phosphorylation), or stabilizing the levels of the complexes themselves (Boekema and Braun, 2007). However, it has been very difficult to test these ideas, as the factors that regulate supercomplex stability or assembly have remained a mystery. Using bakers' yeast as a model system, three independent studies have now identified some of the protein components that regulate supercomplex assembly and stability (Vukotic et al., 2012; Chen et al., 2012; Strogolova et al., 2012).

As fermentative yeasts lack a functional CI, the only supercomplexes that have been observed are associations of a CIII dimer with a CIV monomer or dimer ($CIII_2/CIV_2$ or $CIII_2/CIV$). Using a tagged version of a CIII structural subunit, the Rehling group affinity purified these supercomplexes, separated them on native gels, and analyzed the components by mass spectrometry (Vukotic et al., 2012). This analysis identified two small, uncharacterized proteins that copurified with the supercomplexes, later named Rcf1 (for respiratory super complex factor 1) and Rcf2. The Stuart group was studying the association of the adenine nucleotide carrier (the protein that transports ATP/ADP in and out of mitochondria) with the supercomplexes and discovered Rcf1 as comigrating protein. Affinity purification of CIII using a tagged structural subunit confirmed the association of Rcf1with the supercomplex (Strogolova et al., 2012). Finally, the Rutter group (Chen et al., 2012) identified Rcf1 in their investigations of phylogenetically conserved but uncharacterized mitochondrial proteins. They affinity purified a tagged version of Rcf1 and, using mass spectrometry analysis, identified several CIII and CIV structural subunits, suggesting that the protein was a component of the supercomplex (Chen et al., 2012).

All three groups found that Rcf1 was tightly associated with CIV, but analysis of a panel of mutants affecting either CIII or CIV assembly showed that it could stably associate with either complex alone, demonstrating that it is not a subunit of CIV and suggesting that it likely bridges a gap between the two complexes. What does Rcf11 have to do with the assembly of the supercomplexes, and what happens when it is missing? The most striking molecular phenotype in Rcf1-deleted cells was a severe reduction in the level of the $CIII_2/CIV_2$ supercomplex. This was accompanied by a specific decrease in CIV activity, decreased oxygen consumption, impaired growth on nonfermentable substrates, and an increase in the production of reactive oxygen species. These phenotypes were exacerbated when both factors were deleted together (Strogolova et al., 2012).

One might have expected a complete dissociation of CIII and CIV and not just a decrease in one species, were Rcf1 both necessary and sufficient for supercomplex stability, and this observation led the Rehling group to test whether in fact all CIV monomers were created equal. These experiments led them to the quite unexpected observation that RCf1 was crucial for the incorporation of a peripheral CIV subunit (Cox13, human COXVIa) and that monomers lacking this subunit could not assemble into the $CIII_2/CIV_2$ complex. Another peripheral CIV subunit (Cox12, human COXVIb) was also inefficiently incorporated when supercomplex assembly was disrupted (Strogolova et al., 2012). Thus, an additional role for supercomplexes may be to fine-tune the cell for growth in response to different metabolic cues through the assembly of different protein isoforms.

Rcf1 is a member of the hypoxia inducible gene 1 (HIG1) family, and it has at least two human homologs, *HIGD1A* and *HIGD2A*. Human HIGD2A partially suppressed the phenotype caused by deletion of the yeast Rcf1 (Vukotic et al., 2012), and siRNA-mediated knockdown of the murine homolog of HIGD2A resulted in a decrease in supercomplexes containing CIV (Chen et al., 2012), suggesting that the mammalian protein is also important in stabilizing CIV-containing supercomplexes. The organization of

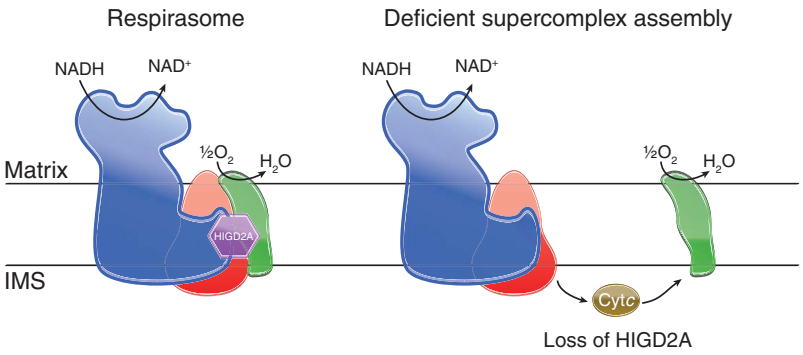

Figure 1. Mammalian Respiratory Chain Supercomplexes

The respirasome is embedded in the inner mitochondrial membrane with components facing the mitochondrial matrix and the intermembrane space (IMS). It contains the mobile electron carriers ubiquinone and cytochrome *c* (Cytc) necessary to transfer electrons between CI and CIII and between CIII and CIV, respectively. It can thus catalyze all of the reactions in the respiratory chain, in which electrons donated from NADH are ultimately used to reduce molecular oxygen. The assembly of the respirasome (C1/$CIII_2$/CIV) requires the activity of HIGD2A, a homolog of Rcf1, which mediates $CIII_2$/CIV_2 supercomplex assembly in yeast. RNAi-mediated knockdown of HIGD2A leads to loss of supercomplexes containing CIV. Under those conditions, reduced Cyt*c* must diffuse to its binding site on free CIV. CI is colored blue, $CIII_2$ red, and CIV green. It seems likely that additional supercomplex assembly factors, mediating the CI/CIII interaction, will be found in mammals.

supercomplexes in mammals is, however, considerably more complex than in yeast, partly because mammals have a CI and because the relative ratios of CI:CIII:CIV are roughly 1:3:6 (Schägger and Pfeiffer, 2001). In a variety of cell lines and tissues, CI appears in a large supercomplex with a CIII dimer and a CIV monomer (C1/$CIII_2$/CIV), the so-called respirasome (Schägger and Pfeiffer, 2001; Moreno-Lastres et al., 2012). Respiratory active respirasomes containing ubiquinone and cytochrome *c* have been isolated (Acín-Pérez et al., 2008), and the placement of substrate binding sites in the structure, determined by CryoEM, permits efficient movement of quinones between CI and CIII and of cytochrome *c* between CIII and CIV (Althoff et al., 2011; Dudkina et al., 2011). CI has been suggested to provide the scaffold for respirasome assembly (Moreno-Lastres et al., 2012), and patients with mutations in CIII and CIV assembly factors often have reduced amounts of CI, further indicating that the respirasome is necessary for CI stability (Moreno-Lastres et al., 2012). On the other hand, substantial fractions of CIII and CIV do not assemble into the respirasome in human cells, but appear as smaller complexes or individual complexes, and defects in CI assembly rarely lead to pleiotropic respiratory chain phenotypes.

What regulates respirasome formation, and why do some complexes apparently not assemble as supercomplexes? Are additional factors necessary to assemble the mammalian respirasome? Is regulation tissue specific, responding to energy demand or nutrient or oxygen supply? Five of the subunits of mammalian CIV (COXIV, VIa, VIb, VIIa, and VIII) have tissue-specific isoforms whose function is not well understood, and perhaps, as in yeast, different supercomplexes could contain different CIV isoforms. Although many questions remain, the identification of the first supercomplex stability factors will now permit manipulation of these structures in animal models to rigorously investigate their function in health and disease.

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