Abstract

Mitochondria, ‘the powerhouses of the cell’, house the integral metabolism pathway of oxidative phosphorylation to produce the majority of cellular energy. Mammalian cytochrome *c* oxidase, also called complex IV (cIV), is indispensable for the overall oxidative phosphorylation function as the terminal oxidase, and for its regulation to sustain energetic needs. Since cIV is a multimeric enzyme composed of subunits encoded by nuclear and mitochondrial genomes, its biogenesis is a complicated process, which needs to be coordinated to complete a fully functional complex. Further, the setup of individual nuclear-encoded subunits isoforms of cIV may fine-tune cIV function based on the tissue or the environment context. Despite the physiological and pathological relevance of cIV composition, biogenesis, and the secondary deficiencies triggered by cIV defects, nuclear-encoded subunits’ function remains poorly understood.

At first, mammalian COX4 subunit isoforms with tissue- and oxygen-dependent expression were studied in the HEK293 cellular model with an exclusive expression of COX4I1 or COX4I2 isoform. Remarkably, the COX4I2 isozyme showed lower affinity to oxygen, which may imply regulation of cIV activity under hypoxia, and is of physiological relevance for the oxygen-sensing mechanism.

Further, analysis of the cIV assembly process utilizing COX4 and COX6B lacking cells approved the indisputable role of the COX4 subunit in the cIV assembly initiation. Unexpectedly, COX6B subunit absence led to complete cIV deficiency with the MT-CO1 module being preserved. This reflects COX6B’s importance not only in the later stages of assembly but also in the stabilization of early assembly intermediates. Moreover, results obtained in this study supported the non-canonical assembly route of cIV subunits directly into the respirasome, which still represents a highly uncharacterized process.

Finally, COX4 and COX6B lacking cells served as ideal models to explore the interdependence of cIV and complex I maintenance, and the respective signaling. cIV deficiency triggered complex I content decrease, presumably through the mitochondrial protein synthesis attenuation. Functional replacement of cIV by alternative oxidase in cIV deficient cells improved its phenotype, indicating functional rather than a structural trigger of cIV and cI interdependence.

Mitochondrial integrated stress response was active in cIV deficient models, being a highly probable signaling pathway in interdependence.

To summarize, the present study explored the role of cIV subunits COX4 and COX6B on multiple levels of its impact on cIV itself, and the oxidative phosphorylation system in general.

Keywords

oxidative phosphorylation, cytochrome *c* oxidase, COX4, COX6B, isoforms, assembly, supercomplexes, interdependence, integrated stress response