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Review

Mitochondrial cytochrome *c* oxidase biogenesis: Recent developments

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ABSTRACT

Mitochondrial cytochrome *c* oxidase (COX) is the primary site of cellular oxygen consumption and is essential for aerobic energy generation in the form of ATP. Human COX is a copper-heme A hetero-multimeric complex formed by 3 catalytic core subunits encoded in the mitochondrial DNA and 11 subunits encoded in the nuclear genome. Investigations over the last 50 years have progressively shed light into the sophistication surrounding COX biogenesis and the regulation of this process, disclosing multiple assembly factors, several redox-regulated processes leading to metal co-factor insertion, regulatory mechanisms to couple synthesis of COX subunits to COX assembly, and the incorporation of COX into respiratory supercomplexes. Here, we will critically summarize recent progress and controversies in several key aspects of COX biogenesis: linear *versus* modular assembly, the coupling of mitochondrial translation to COX assembly and COX assembly into respiratory supercomplexes.

Contents

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1. Introduction

One of the defining characteristics of a living system is the ability of many translated proteins to fold and assemble into macromolecular complexes with precision and fidelity. Assembly is particularly complicated for hetero-multimeric complexes, for which the process often requires the assistance of dedicated chaperones and undergoes multiple quality control checkpoints. It is, therefore, not surprising that the failure of complex-components to fold or assemble correctly is the origin of a wide variety of pathological conditions [1]. Uncovering the mechanisms through which such assembly processes take place is one of the grand challenges of modern science from a biological and a biomedical perspective.

Within mitochondria, accurate assembly processes are fundamental for the building of the macromolecular complexes that provide the backbone of aerobic energy production in eukaryotes by the oxidative phosphorylation (OXPHOS) system. The biological roles of these complexes are relevant to cellular adaptation to changing environments as well as to multiple disease scenarios, including encephalo- and cardio-myopathies, cancer, neurodegeneration and the aging process [2]. From a biogenetic point of view, a distinctive level of convolution impinges the assembly of the four OXPHOS complexes whose components are derived from both the mitochondrial and the nuclear genomes. As a case in point, in this manuscript we will discuss the biogenesis of the mitochondrial respiratory chain (MRC) terminal oxidase, the cytochrome *c* oxidase (COX) enzyme or MRC complex IV (CIV).

1.1. Mammalian COX components, evolution and assembly factors

COX is a copper-heme A terminal oxidase embedded in the mitochondrial inner membrane [3]. It catalyzes electron transfer from reduced cytochrome *c* to molecular oxygen in a process coupled to the transfer of protons across the inner membrane, thus contributing to the generation of the proton gradient that is used by ATP synthase to drive ATP synthesis. Mammalian COX is a multimeric enzyme formed by 14 subunits of dual genetic origin. The three subunits forming the catalytic core of the enzyme (COX1, COX2 and COX3) are encoded by the mitochondrial DNA, and the remaining by the nuclear DNA. The structure of the core subunits is conserved from α-proteobacteria, the ancestors of mitochondria, to human COX. COX1 and COX3 are highly hydrophobic, integral membrane proteins with no substantial extramembrane domains. COX2, on the other hand, consists of a β barrel extramembrane domain anchored by two transmembrane helices that bind to COX1, opposite from the side of COX1 that binds COX3 (Fig. 1**A**). Subunits COX1 and COX2 contain the redox metal active centers of the enzyme. The extramembrane domain of COX2 extends into the mitochondrial intermembrane space (IMS) to bind soluble cytochrome *c*. A di-copper center termed Cu_A accepts the electrons from soluble cytochrome *c*. The Cu_A center is located in a loop region at the bottom of the extramembrane domain of COX2, at the interface of COX1 and COX2 (Fig. 1**B**). From Cu_A, electrons flow to heme *a* in COX1, which transfers the electrons to the heme a_3-Cu_B binuclear center where O_2 is reduced to water (Fig. 1**B**) [3].

Through evolution, up to eleven smaller subunits (in mammals) have been added to the catalytic core (Fig. 1**A**). This has presumably allowed to cope with evolving energetic requirements of eukaryotic cells over time, to adapt to oxygen-rich environments, and to regulate complex IV activity to coordinate energy production with timely cellular needs [4]. The last subunit of the mammalian complex to be identified was NDUFA4 [5]. NDUFA4 was absent from the crystal structures [6] and had been mistakenly assigned as a subunit of complex I. Adding convolution, mammalian COX has multiple tissue-specific isoforms of nuclear encoded subunits, which portraits the key regulatory role COX has on OXPHOS [4]. Assembly of functional COX is not trivial, since the process is complicated by the dual genetic origin of the COX subunits. Hence, it is not surprising that eukaryotic cells have evolved nucleus-encoded assembly factors and signaling pathways to coordinate mitochondrial-nuclear expression of COX genes, to control the abundance of the subunit and metal cofactor components, and to direct their assembly into a functional enzyme [7,8]. Already the four-subunit aa_3-type COX of α-proteobacteria requires the assistance of five dedicated conserved assembly proteins for the accumulation of the complex in the membrane. These are COX10 and COX15 that function to synthesize heme A, SURF to possibly deliver heme A, SCO to assemble Cu_A, and COX11 to assemble Cu_B, as it will be detailed in the following sections.

Mitochondria contain more than 30 COX assembly factors (Table 1), a list that continues to be extended. For example, immunocapture of COX assembly intermediates has facilitated the identification of the short isoform of the myofibrillogenesis regulator 1 (MR-1S) as a protein that works with the conserved PET100 and PET117 chaperones to assist late stages of COX biogenesis in higher eukaryotes [9]. Over the last 50 years, several model organisms and experimental systems have been used for COX assembly studies. These studies have produced a substantial amount of information regarding the individual assembly factors, some mechanistic detail, and numerous hypotheses and models of the assembly processes. These, along with recent developments and standing controversies, will be discussed in this article, with a primary focus on the mammalian enzyme.

1.2. *Linear* vs. *modular COX assembly*

Since the pioneering studies on COX assembly during the early 1980s, the process was thought to be linear, with the different subunits and cofactors being added in a sequential and ordered manner. This concept was based on studies that used rat liver mitochondria and followed the incorporation of radiolabeled subunits into the COX holocomplex [10]. The model was later confirmed by analyzing the formation of assembly intermediates in human cultured cells by Blue-Native electrophoresis, which allowed researchers to conclude that *de novo* assembly initiates around a seed formed by COX1 and proceeds with the formation of several discrete assembly intermediates that accumulate at rate-limiting steps in the process [11,12].

The concept of linear assembly requires modification, however. Studies using the yeast *Saccharomyces cerevisiae* have shown that the biogenesis of each of the three subunits of the catalytic core proceeds by a relatively independent process with the participation of subunit-specific chaperones [13]. This promotes the idea of COX assembly as a modular process. Consistent with this concept, the COX catalytic core subunits in human cells also appear to form preassembly modules, which in the case of COX1 and COX2 are fundamental to ensure proper subunit maturation by the incorporation

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