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Review

Cytochrome *c* oxidase: Evolution of control via nuclear subunit addition ☆

Denis Pierron, Derek E. Wildman, Maik Hüttemann, Gopi Chand Markondapatnaikuni, Siddhesh Aras, Lawrence I. Grossman *

Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48201, USA

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ABSTRACT

According to theory, present eukaryotic cells originated from a beneficial association between two free-living cells. Due to this endosymbiotic event the pre-eukaryotic cell gained access to oxidative phosphorylation (OXPHOS), which produces more than 15 times as much ATP as glycolysis. Because cellular ATP needs fluctuate and OXPHOS both requires and produces entities that can be toxic for eukaryotic cells such as ROS or NADH, we propose that the success of endosymbiosis has largely depended on the regulation of endosymbiont OXPHOS. Several studies have presented cytochrome *c* oxidase as a key regulator of OXPHOS; for example, COX is the only complex of mammalian OXPHOS with known tissue-specific isoforms of nuclear encoded subunits. We here discuss current knowledge about the origin of nuclear encoded subunits and the appearance of different isozymes promoted by tissue and cellular environments such as hypoxia. We also review evidence for recent selective pressure acting on COX among vertebrates, particularly in primate lineages, and discuss the unique pattern of co-evolution between the nuclear and mitochondrial genomes. Finally, even though the addition of nuclear encoded subunits was a major event in eukaryotic COX evolution, this does not lead to emergence of a more efficient COX, as might be expected from an anthropocentric point of view, for the "higher" organism possessing large brains and muscles. The main function of these subunits appears to be "only" to control the activity of the mitochondrial subunits. We propose that this control function is an as yet underappreciated key point of evolution. Moreover, the importance of regulating energy supply may have caused the addition of subunits encoded by the nucleus in a process comparable to a "domestication scenario" such that the host tends to control more and more tightly the ancestral activity of COX performed by the mtDNA encoded subunits. This article is part of a Special Issue entitled: Respiratory Oxidases.

1. Introduction

Lynn Margulis' theory about a prokaryotic origin for eukaryotic mitochondria is now broadly accepted [1–3]. According to this theory, present eukaryotic cells originated from a beneficial symbiosis between two free-living cells. Indeed, an α-proteobacterium was supposedly taken inside the pre-eukaryotic (host) cell and then formed an obligate endosymbiont.

Due to symbiotic association with the endosymbiont, i.e., the mitochondrial ancestor, the host cell gained access to oxidative phosphorylation (OXPHOS), which generates an ATP yield that is more than 15 times higher than glycolysis. With the exception of some parasitic organisms (such as *Giardia* or microsporidia), the conservation of OXPHOS across most eukaryotic lineages (e.g., plants, animals, fungi) suggests that this system is crucial for eukaryotic life. However, besides ATP, OXPHOS also produces reactive oxygen species (ROS, also sometimes referred to as 'free radicals') and heat, and requires oxygen and nutrients. Too large or too small an amount of these substrates and products can be toxic for eukaryotic cells. For example, an excess of substrate such as NADH can lead to lactic acidosis by driving lactate dehydrogenase to produce lactate [4] whereas an excess of products or by-product such as ROS can lead to apoptosis [5]. OXPHOS activity, therefore, has to be adjusted to take into account supply of nutrients and demand for energy. Furthermore, energy requirements differ among different cells from the same eukaryotic organism and from the same cell during its lifespan. All things considered, we propose that the success of endosymbiosis has largely depended on the regulation of OXPHOS activity, implying tight host-endosymbiont communication.

Several studies have presented complex IV (cytochrome *c* oxidase, COX, EC 1.9.3.1) as a key regulator of overall respiratory chain activity in intact mammalian cells: (i) COX has a high control coefficient *in vivo* on OXPHOS activity, meaning a decrease of COX activity decreases ATP production [6–8]; (ii) expression, assembly, and activity of COX were shown to be highly regulated [9,10]; and (iii) intrinsic biochemical parameters of COX were shown to be tissue specific [11] due to different isoform expression; for example, liver-type COX, which is expressed in tissues that rely fully on aerobic

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* Corresponding author. Tel.: +1 313 577 5326; fax: +1 313 577 5218.
E-mail address: l.grossman@wayne.edu (L.I. Grossman).

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energy metabolism but cannot spare more room to increase the mitochondrial complement, has a higher basal activity compared to skeletal muscle/heart-type COX [11–16].

Mitochondrial encoded subunits carry out both electron transfer and proton-pumping functions, but it has been proposed that these enzymatic activities are mainly regulated through the nuclear encoded subunits [17]. Here, we discuss how evolutionary events that adapted OXPHOS activity to cellular requirements increased the fitness of the two genomes and were then positively selected and conserved. The importance of regulating energy supply may have caused a process comparable to a "domestication scenario" such that the host tends to control more and more tightly the ancestral activity of COX performed by the mtDNA encoded subunits through the addition of subunits encoded by the nucleus.

After a brief summary of our current understanding about the electron transfer and proton-pumping functions, we discuss the origin of nuclear encoded subunits and the appearance of different isozymes promoted by tissue and cellular environments such as hypoxia. Finally, we review evidence for recent selective pressure acting on COX among vertebrates, particularly in primate lineages, and discuss the unique pattern of co-evolution between the nuclear and mitochondrial genomes.

2. Ancestral function of cytochrome *c* oxidase

The mitochondrial respiratory chain couples the reduction of molecular oxygen to the translocation of protons across the inner mitochondrial membrane [18]. In mammals, the first step of the respiratory chain is the oxidation of NADH or $FADH_2$ by, respectively, complexes I and II, followed by electron transfer to complex III via coenzyme Q, and finally transfer via cytochrome *c* to complex IV (COX), which reduces the final acceptor oxygen to water. Complexes I, III, and IV couple the redox reactions to the translocation of protons across the inner mitochondrial membrane. These translocations generate a proton gradient that permits ATP synthase to synthesize ATP from ADP and inorganic phosphate.

Mitochondrial COX, the terminal complex of the respiratory chain, belongs to a large family of heme-copper terminal oxidases, also containing prokaryotic aa_3-type COX. Although mammalian mitochondrial COX has 13 subunits and prokaryotic aa_3-type has only three to four subunits [19,20], the amino acid sequences of the core subunits I and II of both enzymes are more conserved than is typical between bacteria and mammalian homologs. For example, there is 52% identity between *Bos taurus* and *Paracoccus denitrificans* subunits I (69% similarity) and 34% identity between subunits II (59% similarity). The X-ray structures of *P. denitrificans* and bovine enzymes confirmed that the structure is also very similar between prokaryotic and mitochondrial COX [21–24]. Although the proton pumping mechanism is not yet fully understood, the different models proposed are consistent with both the mitochondrial and prokaryotic structures.

In mammalian mitochondria COX is embedded in the inner membrane and reduced cytochrome *c* binds to COX on the intermembrane space side [17]. Electrons from cytochrome *c* are passed through the Cu_A center located in subunit II (Fig. 1), then go to subunit I, from heme *a* to the heme a_3/Cu_B center. This is the catalytic center of the enzyme, which reduces oxygen to water, by consuming electrons that come initially from cytochrome *c* and four chemical protons that are taken up from the matrix. This reaction is exergonic and is coupled to the translocation of four additional protons from the matrix to the intermembrane space. The proton gradient generated powers the conversion of ADP and phosphate to ATP by ATP synthase [25]. At least two pathways are currently thought to conduct protons inside subunit I [26–29]: (i) the K pathway (via Lys319, according to bovine numbering), which leads to the heme a_3/Cu_B (catalytic center), and (ii) the D pathway (via Asp91 to Glu242, according to bovine numbering), where protons can be directed to the heme a_3/Cu_B center and/or to the intermembrane space.

As prokaryotic subunits I and II are sufficient to carry out the electron transfer and proton-pumping functions, COX presumably has been functional since the initiation of endosymbiosis. However, because the energetic needs of eukaryotic cells undoubtedly changed over time, selective pressures would have rapidly appeared to favor synchronization of energy production to those cellular needs.

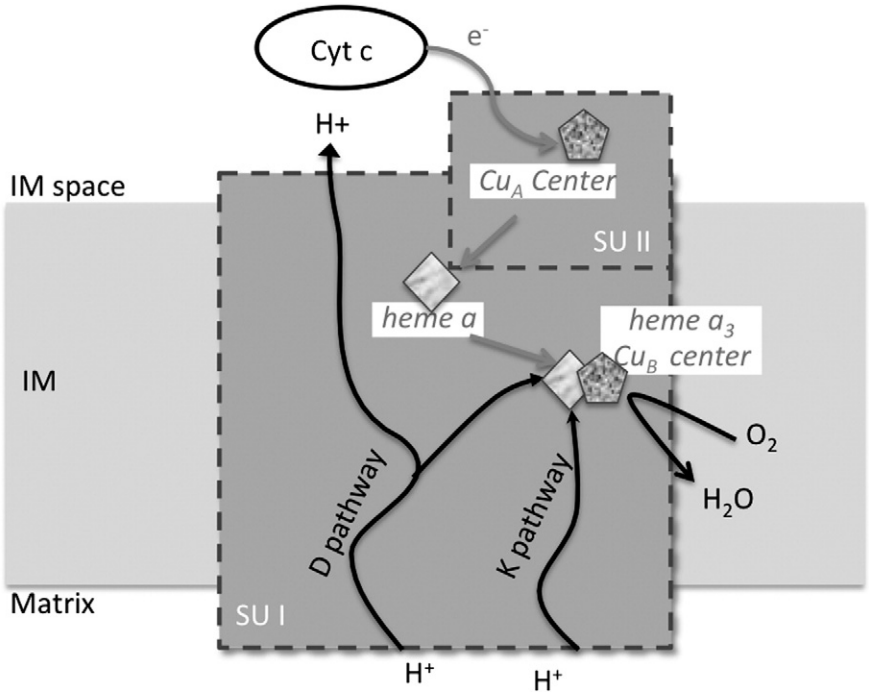

Fig. 1. Mechanism of cytochrome *c* oxidase, squares symbolize heme and pentagons symbolize coppers. IM space, intermembrane space; IM, inner membrane; gray arrows represent electron pathway and black arrows represent proton transfer pathway and oxygen reduction.