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Review

Biogenesis of cytochrome oxidase—Sophisticated assembly lines in the mitochondrial inner membrane

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Abstract

Biogenesis of the cytochrome oxidase complex in the mitochondrial inner membrane depends on the concerted action of a variety of proteins. Recent studies shed light on this biological assembly process revealing an astonishingly complex procedure by which the different subunits of the enzymes are put together and the required cofactors are supplied. In this review we present a hypothetical model for the assembly process of cytochrome oxidase based on the current knowledge of the functions of specific assembly factors. According to this model the two largest subunits of the complex are first equipped with their respective cofactors on independent assembly lines. Prior to their assembly with the residual subunits that complete the whole complex, these two subcomplexes remain bound to substrate-specific chaperones. We propose that these chaperones, Mss51 for subunit 1 and Cox20 for subunit 2, control the coordinate assembly process to prevent potentially harmful redox reactions of unassembled or misassembled subunits.

Keywords: Complex IV; Mitochondria; Protein assembly; Reactive oxygen species; Respiratory chain

1. Introduction

The cytochrome oxidase is the terminal enzymatic complex of the respiratory chain in eukaryotes. It couples the transfer of electrons between cytochrome *c* and molecular oxygen to the vectorial translocation of protons across the inner membrane of mitochondria. This central role in the cellular energy production makes cytochrome oxidase a component of fundamental importance for aerobic organisms. In recent years, the biogenesis of the cytochrome oxidase complex gained much interest because defects in the assembly of the enzyme are a major cause of mitochondrial disorders in humans and may play an important role in aging and degenerative diseases (for review see Schon, 2000; Shoubridge, 2001; Smeitink et al., 2001; Wallace, 2001).

The mitochondrial cytochrome oxidase complex of yeast and mammals consists of 12 and 13 subunits, respectively. The crystal structure of the bovine enzymes was solved allowing detailed insights into the three-dimensional organisation of the complex (Tsukihara et al., 1996). The three largest subunits, Cox1, Cox2 and Cox3, form the catalytic core of the enzyme. These subunits are largely embedded into the inner membrane and are encoded by mitochondrial DNA. All three proteins are also present in the bacterial cytochrome oxidase complex and have been highly conserved throughout evolution. The heme and copper cofactors, which take part in the electron transport activity of cytochrome oxidase, are coordinated by Cox1 and Cox2. Several simply structured accessory subunits are associated with the hydrophobic core. These subunits are absent in bacteria and show mostly low sequence conservation among different eukaryotic species. They were most likely acquired during evolution of the eukaryotic cell and are encoded by nuclear genes. The function of these accessory subunits is

Abbreviations: EST, Expressed sequence tags; NCBI, National Center for Biotechnology Information; UTR, Untranslated region.

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not well understood and they may be predominantly involved in the stabilization and regulation of the complex and in physical interactions with other enzymes of the respiratory chain.

2. The assembly line for cytochrome oxidase

Over the last three decades, genetic screens in the yeast *Saccharomyces cerevisiae* and other fungi identified a plethora of gene products which in addition to the structural subunits are required for cytochrome oxidase activity (McEwen et al., 1986; Tzagoloff and Dieckmann, 1990; Barrientos et al., 2002). Numerous biochemical studies allowed it to assign these components to specific biological processes, like transcription (Shadel, 2004), processing (Seraphin et al., 1988, 1989), and translation (see Section 3) of the mitochondrial mRNAs, synthesis of the heme cofactor and copper incorporation. The known components required for heme and copper insertion were described in detail in recent review articles (Barrientos et al., 2002; Barros and Tzagoloff, 2002; Carr and Winge, 2003). The residual factors were mostly referred to as 'assembly factors', based on the fact that the absence of these proteins abolishes the activity of the enzyme but they are not present in the mature complex. However, the molecular function of these components is poorly understood.

A sequential order of the assembly process of cytochrome oxidase was proposed more than 20 years ago on the basis of co-immunoprecipitation experiments of pulse-labeled cell cultures (Wielburski and Nelson, 1983). The precise order of events and the individual roles of the assembly factors, however, remain largely obscure due to a number of technical difficulties: (1) The location of the genes of the core subunits on the mitochondrial genome impedes their manipulation by standard methods of molecular biology. (2) Cox1 and Cox3 are extremely hydrophobic; they tend to aggregate when released from the membrane with detergents and to undergo artificial post-lysis interactions. (3) Assembly intermediates of cytochrome oxidase are proteolytically unstable. Hence, the complexes isolated from assembly mutants do not necessarily represent accumulating stages in the biogenesis of the enzyme but rather partially stable degradation products. (4) Assays in which the assembly of the complex could be monitored in vitro or in isolated mitochondria could not be developed thus far. (5) The insertion of cofactors requires a number of different gene products and experimental assays to follow the acquisition of copper ions and heme groups are largely missing.

Nevertheless a number of recent studies by several laboratories allow exciting insights into the assembly process of this complicated enzyme. Here, we will try to summarize the current knowledge on the biogenesis of cytochrome oxidase and propose a hypothetical model for its assembly process. This process resembles industrial assembly lines in which various components perform sequential operations on the individual mitochondrially encoded subunits of the enzyme and which finally merge to build the active complex from the different parts (Fig. 1).

3. Protein synthesis and membrane integration

3.1. Translational activators bind to mRNAs

Cox1, Cox2 and Cox3 are encoded by mitochondrial genes and synthesized on mitochondrial ribosomes. In yeast mitochondria, translational activators bind the 5′ untranslated leaders of mRNAs and tightly regulate the levels of protein synthesis (Fox, 1996a,b). Moreover, these activators also interact with the RNA polymerase via Nam1 thereby coupling transcription and translation processes (Rodeheffer et al., 2001). Each mitochondrial gene appears to have its own set of regulating factors. The synthesis of Cox1 is under control of Pet309 and Mss51 (Manthey and McEwen, 1995; Manthey et al., 1998), Cox2 translation is regulated by Pet111 (Mulero and Fox, 1993a,b), and Cox3 by Pet54, Pet122 and Pet494 (Costanzo and Fox, 1988; Brown et al., 1994). These translational activators are associated with the

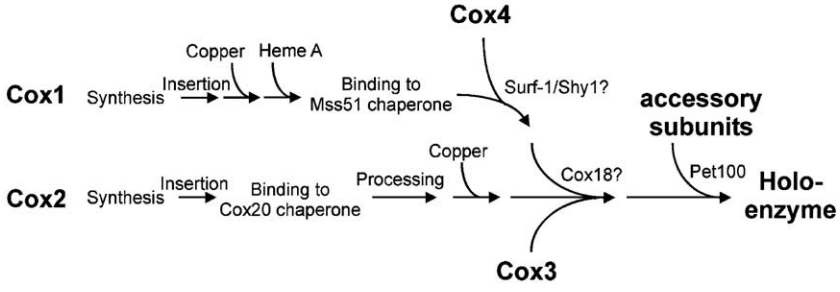

Fig. 1. Model for the assembly process of cytochrome oxidase. Following their synthesis and integration into the inner membrane, Cox1 and Cox2 are individually modified and equipped with their respective cofactors in several reactions. These reactions appear to occur in a sequentially ordered manner forming two parallel assembly lines which finally merge to form a core complex. Substrate-specific chaperones bind to Cox1 and Cox2, thereby maintaining these proteins in an assembly-competent state. Upon association of the residual nuclear encoded subunits with the core complex, the final holoenzyme is formed and the assembly reaction completed. The formation of the core complex requires the function of Surf-1/Shy1 and Cox18/Oxa2; Pet100 plays an important role in a late stage of the assembly reaction. See text for details.