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Review

Function and redox state of mitochondrial localized cysteine-rich proteins important in the assembly of cytochrome *c* oxidase

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Abstract

The cytochrome *c* oxidase (CcO) complex of the mitochondrial respiratory chain exists within the mitochondrial inner membrane (IM). The biogenesis of the complex is a multi-faceted process requiring multiple assembly factors that function on both faces of the IM. Formation of the two copper centers of CcO occurs within the intermembrane space (IMS) and is dependent on assembly factors with critical cysteinyl thiolates. Two classes of assembly factors exist, one group being soluble IMS proteins and the second class being proteins tethered to the IM. A common motif in the soluble assembly factors is a duplicated Cx_9C sequence motif. Since mitochondrial respiration is a major source of reactive oxygen species, control of the redox state of mitochondrial proteins is an important process. This review documents the role of these cysteinyl CcO assembly factors within the IMS and the necessity of redox control in their function.

Keywords: Cytochrome oxidase; Copper; Mitochondria; Intermembrane space

1. Mitochondrial organization

The mitochondrion consists of a continuous reticulum that makes up nearly 10% of the cell volume in respiring yeast cells. The tubular network is highly dynamic and changes size and shape through fission and fusion events [1]. A double membrane forming two internal spaces encloses the mitochondria. The space between the two membranes is called the intermembrane space (IMS) and the volume enclosed within the inner membrane (IM) is designated as the matrix compartment. The two membranes differ significantly in their composition with the IM more highly enriched in protein content. The outer membrane (OM) envelope contains 25 Å nm pores that allow diffusion of small ions such as glutathione. The IM is a barrier to diffusion, so the passage of metabolites requires transporters. As such, the import of glutathione across the IM is believed to occur through dicarboxylate and 2-oxoglutarate transporters [2].

Mitochondria maintain their genome within the matrix compartment. This genome codes for a limited number of polypeptides, 13 and 8 in humans and yeast, respectively. All, but one, of these proteins are components of the oxidative phosphorylation system.

The double membrane of mitochondria is interrupted by junction points of contact between the IM and OM [3]. The junction points are likely formed by protein assemblies involved in protein import [4,5]. The mean distance across the OM and IM is 20 nm, although the distance narrows to only about 14 nm at junction points. In cells with high respiration rates, the IM is invaginated, folding into tubular structures designated cristae. Cristae are enriched in the enzyme complexes involved in oxidative phosphorylation. Electron microscopy tomography revealed that the cristae tubules are 30–40 nm in diameter but narrow to about 28 nm at junction points with the boundary IM, designated inner boundary membrane (IBM) [3,5]. The constriction of the cristae junctions at the IBM resolves the soluble IMS into separate volumes that appear to be in equilibrium only for small molecules [6]. Cristae can exist as tubular structures or merged to form flattened lamellar compartments. Stacked lamellar cristae remain connected to the IBM by tubular cristae

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junctions. The cristae junctions are believed to be dynamic and modulated by the energetics of the organelle as well as by the fusion/fission process.

In this review, the lumen of the cristae will be referred to as the cristae lumen and the space between the boundary IM and OM as the boundary IMS. The generic term IMS will specify the generalized volume between the OM and IM without subcompartmentization specification.

Oxidative phosphorylation occurs predominantly, but not exclusively, on the cristae membrane (CM). The enrichment of cytochrome *c* oxidase (CcO) in cristae is well established [7], but recent studies confirm the abundance of complexes III (cytochrome bc_1 complex) and V (ATP synthase) within the CM [5]. Respiratory complexes I, III and IV are largely present as supercomplexes within the CM [8–11]. The yeast III/IV supercomplex consists of a dimeric complex III species at the core with one or two CcO complexes at opposite ends [11]. Synthesis and assembly of the respiratory complexes occurs preferentially on the CM as mitochondrial ribosomal proteins are associated with the CM [5].

2. Generation of reactive oxygen species in mitochondria

Mitochondrial respiration is a major source of reactive oxygen species. Respiring mitochondria convert 1–2% of the oxygen consumed to superoxide anion [12,13]. The bulk of superoxide anion produced on a daily basis in most organisms comes from ubisemiquinone of coenzyme Q within the respiratory chain [14]. CoQ shuttles electrons from complexes I and II to complex III. The CoQ semiquinone generated either at complex I or during the Q-cycle in complex III can react with oxygen generating superoxide anions. Based on the sidedness of the Q-cycle in complex III, superoxide is generated in both the matrix and IMS, although the bulk of the superoxide is generated within the matrix [15,16]. Superoxide may itself cause damage or may react further to yield other reactive species such as hydrogen peroxide or the hydroxyl radical. The presence of reactive oxygen species can induce oxidation events such as modification of protein thiols on both sides of the IM [17]. Endogenous reactive oxygen species generated at complex I and III were shown to modify nine distinct mitochondrial proteins [17]. Several of the modified proteins function in fatty acid oxidation.

The normal production of superoxide anion during respiration and subsequent generation of hydrogen peroxide and other oxidants in both the IMS and matrix necessitates control of transition metal ion availability to minimize Fenton chemistry to generate more potent oxidants. Copper ions used in metallation reactions are protein bound to minimize the deleterious effects of unbound Cu(I) ions. Copper metallation of CcO and superoxide dismutase (Sod1) within the IMS occurs by metallochaperone proteins Cox17 and Ccs1, respectively. Only a small fraction of the cellular Sod1 exists within the IMS [18]. The transfer reactions are protein-mediated. However, the Cu(I) sites on Ccs1 are partially solvent accessible and this is likely also true for Cox17. Although the level of copper complexes of these two proteins isn't known within the IMS, it is possible that the level of Cu(I) within the IMS may be regulated to minimize chances of copper-induced oxidation reactions. The Cu(I) ions used in the IMS metallation reactions derives from a storage pool within the matrix [19,20]. It is conceivable that the Cu(I) transporter within the IM that translocates Cu(I) to the IMS is regulated such that the Cu(I) transported is coupled to the biogenesis of CcO and Sod1.

Another defense against deleterious oxidative processes is the availability of redox systems to maintain redox homeostasis. The effectiveness of the matrix redox system is highlighted by the observation that the apparent redox potential of the mitochondrial matrix is more negative (−360 mV) relative to the cytoplasm (−320 mV) in HeLa cells using redox-sensitive fluorescent GFP variants [21,22]. No information is available on the redox potential of the IMS compartment.

The mitochondria matrix contains well-defined redox components including glutathione, thioredoxin and glutaredoxin systems. A fraction of the cellular glutathione reductase Glr1 exists within the matrix in yeast. The thioredoxin system involves the mitochondrial-specific Trx3 thioredoxin and the Trr2 thioredoxin reductase. Both monothiol (Grx5) and dithiol (Grx2) glutaredoxins exist within the matrix. Initiation at an upstream ATG in the transcripts for Grx2 and Glr1 generate the mitochondrially-targeted variants. The presence of multiple protein reductants and the overlapping functions of Glr1 and Trr2 reductases illustrates that a robust redox pathway exists within the matrix. Apart from an initial report of mammalian thioredoxin reductase-I existing within the IMS [23], it is not clear whether other redox components are present within the IMS compartments. The porous OM suggests that GSH may equilibrate across the membrane, but no evidence exists for the IMS presence of Glr1 to maintain GSH/GSSG redox homeostasis.

3. Role of cysteines in IMS proteins

The mitochondrial proteome is expected to contain nearly 850 proteins in yeast [24] but closer to 1000 distinct proteins in humans [25]. About 14% of the proteins are involved in oxidative phosphorylation, whereas 25% are predicted to be involved in maintaining and expressing the mitochondrial genome [26]. A subset of the proteome resides within the IMS either as soluble proteins or as molecules tethered to the IM. Many of these proteins are either cysteine-rich or have functionally important cysteine residues that can exist as within disulfide bonds or as reduced thiolates. The abundance of disulfide-containing molecules within the IMS suggests that redox control within this compartment differs from that within the cytoplasm where disulfide bond formation is rare due to the high reducing potential of the cytoplasm [27]. The constriction of the cristae junctions creating both cristae lumen and the boundary IMS opens the possibility that redox pathways may be distinct within the two subcompartments.

A common structural motif of cysteine-rich proteins within the IMS is a helical hairpin conformer. The structural paradigm for the helical hairpin motif in IMS proteins is small Tim proteins (Tim9/Tim10) characterized by a conserved twin Cx_3C sequence motif. Tim9 and Tim10 each form a helix-loop-helix