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Organelles in focus

Mitochondrial copper homeostasis and its derailment in Wilson disease

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ABSTRACT

In mitochondria, copper is a Janus-faced trace element. While it is the essential cofactor of the mitochondrial cytochrome c oxidase, a surplus of copper can be highly detrimental to these organelles. On the one hand, mitochondria are strictly dependent on adequate copper supply for proper respiratory function, and the molecular mechanisms for metalation of the cytochrome c oxidase have been largely characterized. On the other hand, copper overload impairs mitochondria and uncertainties exist concerning the molecular mechanisms for mitochondrial metal uptake, storage and release. The latter issue is of fundamental importance in Wilson disease, a genetic disease characterized by dysfunctional copper excretion from the liver. Prime consequences of the progressive copper accumulation in hepatocytes are increasing mitochondrial biophysical and biochemical deficits. Focusing on this two-sided aspect of mitochondrial copper, we review mitochondrial copper homeostasis but also the impact of excessive mitochondrial copper in Wilson disease.

1. Introduction

Copper is a trace element, essential for neurotransmitter, neuropeptide and collagen biosynthesis, wound healing, angiogenesis, growth and iron utilization (Kaplan and Maryon, 2016; Owen, 1973). Recently, copper has been suggested to regulate the systemic delivery of triglycerides from the GI tract (Pierson et al., 2017; Weiss and Zischka, 2018). Intracellularly, the two most important copper functions are linked to its redox ability as cofactor of either mitochondrial cytochrome c oxidase (CcO) or of the reactive oxygen species (ROS) detoxifying Cu/Zn superoxide dismutase (SOD1) (Blockhuys et al., 2017). These two enzymes manage the biochemical challenge of a safe copper-mediated reduction/disproportionation of oxygen or ROS, respectively. Unbound “free” copper ions and ROS would otherwise inevitably cause the emergence of hydroxyl radicals that are highly detrimental to proteins, nucleic acids and lipids, via Fenton-based chemistry. Indeed, physiologically, copper ions are not “free”, i.e., dissolved in water, but strictly bound to carrier molecules and distributed intracellularly by so-called copper chaperones to avoid such cellular toxicity (Rae et al., 1999).

Mitochondria harbor the CcO and around 1–5% of total cellular SOD1 and, therefore, are a major site of intracellular copper utilization (Sturtz et al., 2001). Indeed, especially in yeast, these organelles have been suggested to be the intracellular copper store (Yang et al., 2005; Cobine et al., 2004). This view originates from the rationale that increased cellular energetic needs may be met by enhanced mitochondrial oxidative phosphorylation activities and plausibly by elevated CcO and consequently elevated copper amounts (Cobine et al., 2006; Leary et al., 2009a). Thus, in order to meet the basal but also enhanced energetic cellular demand, there is a constant copper supply to mitochondria, and elevated copper loads can be handled by mitochondria (Cobine et al., 2004; Zischka et al., 2011). However, a steadily increasing and excessive mitochondrial copper load may severely affect these organelles. As it is the case in Wilson disease (WD), hepatic copper overload leads to mitochondrial destruction, hepatocyte death and even liver failure. In this article, we focus on current knowledge but also on controversial

Abbreviations: ATP7B, ATPase copper transporting beta; *CcO*, cytochrome c oxidase; *CCS*, copper chaperone for superoxide dismutase; *COX1*, cytochrome c oxidase subunit 1; *COX2*, cytochrome c oxidase subunit 2; *COX11*, cytochrome c oxidase assembly protein 11; *COX17*, cytochrome c oxidase copper chaperone 17; *COX19*, cytochrome c oxidase assembly protein 19; *COX23*, cytochrome c oxidase assembly protein 23; *CuL*, copper ligand; *D-PA*, D-penicillamine; *GI*, gastrointestinal tract; *GSH*, glutathione; *GSSG*, glutathione disulfide; *HEK293*, human embryonic kidney 293 cell line; *IMS*, intermembrane space; K_{Cu}, Cu^{1+}-binding dissociation constant; *LEC*, Long-Evans Cinnamon rat; *LPP*, crossbred from Long-Evans Cinnamon rat and Piebald Virol Glaxo rat; *MFRN1*, mitoferrin 1; *MOM*, mitochondrial outer membrane; *ROS*, reactive oxygen species; *SCO1/2*, synthesis of cytochrome c oxidase proteins 1/2; *SLC25A3*, solute carrier family 25 member 3; *SOD1*, superoxide dismutase 1; *TGN*, trans-Golgi network; *WD*, Wilson disease

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theories about mitochondrial copper homeostasis with a special focus on liver mitochondria. We further outline how a disturbed copper balance induces mitochondrial dysfunction and cellular damage in WD.

2. Mitochondrial copper homeostasis

It has been estimated that a rat liver mitochondrion contains about fifteen to sixteen thousand CcO molecules (Schwerzmann et al., 1986), and that mitochondrially localized SOD1 constitutes around 0.06% of the total mitochondrial protein content (Okado-Matsumoto and Fridovich, 2001). This means that 10^9 mitochondria comprising about 125 µg total protein (Schmitt et al., 2014) would contain around 75 ng SOD1, i.e., about 4.7 pmoles SOD1 (M = 15,943 g/mol) or about 2,800 SOD1 molecules per mitochondrion. Given three copper ions per CcO and one per SOD1, this would amount to around 45,000–50,000 copper atoms per mitochondrion, or around 40 ng/mg mitochondrial protein (assuming $8.1*10^9$ mitochondria per mg of mitochondrial protein (Schmitt et al., 2014)). This value matches reported mitochondrial copper contents of rat liver but also human liver mitochondria ranging from 30 to 50 ng/mg (Zischka et al., 2011; Sokol et al., 1994; Zischka and Lichtmannegger, 2014). As these two mitochondrial copper enzymes are essential for hepatocyte bioenergetics and survival, mitochondria therefore require an adequate copper supply.

The functional mitochondrial copper need is met by copper transporters, so-called copper chaperones (below) and small molecular copper ligands as depicted in Fig. 1. Two prerequisites ensure a safe and robust mitochondrial copper supply. First, in cells, copper is strictly bound to proteins or small molecule ligands to avoid uncontrolled copper redox activity (Rae et al., 1999). Second, the main driving force of copper to be incorporated into CcO and SOD1 is their enormous copper binding affinity (Cu^{1+}-binding dissociation constant K_{Cu} below femtomolar), and an increasing copper affinity of the intermediate copper transporting molecules ensures their directed delivery to CcO and SOD1 (Banci et al., 2010).

As the copper-containing subunits of CcO, COX1 and COX2, are mitochondrially encoded proteins and as metal free apo-SOD1 is imported into the mitochondrial intermembrane space (IMS), copper metalation of these proteins occurs within mitochondria (Field et al., 2003). How is the metal delivered and distributed to and within mitochondria? Most of our current knowledge concerning this issue comes from sophisticated studies in yeast and several, not mutually exclusive, hypotheses have been put forward:

First, copper chaperones, low molecular mass proteins that hand over copper by protein-protein interactions (Banci et al., 2010), have been suggested to transport copper into mitochondria. Indeed, the CcO assembly proteins 19 and 23 (COX19, COX23), as well as COX17, are small soluble proteins containing cysteine residues that bind Cu(I), exhibiting dual localization in cytosol and the IMS (Fig. 1) (Glerum et al., 1996; Nobrega et al., 2002; Barros et al., 2004). However, yeast depleted in these proteins had wild-type mitochondrial copper levels (Cobine et al., 2004; Glerum et al., 1996; Nobrega et al., 2002; Barros et al., 2004). Moreover, CcO deficiency in *cox17Δ*, *cox19Δ* or *cox23Δ* mutant yeast can be restored by external copper supplementation (Glerum et al., 1996; Nobrega et al., 2002; Barros et al., 2004). The same holds true for the dually localized CCS, the SOD1 copper chaperone (Cobine et al., 2004; Field et al., 2003). Thus, while copper chaperones enable mitochondrial CcO and SOD1 activities, alternative mitochondrial copper uptake molecules are likely to be present.

A second potential copper entry or export mechanism to or from the IMS may occur via the tripeptide glutathione (GSH, Fig. 1), as GSH can easily cross the mitochondrial outer membrane (MOM) through porin channels (Mari et al., 2009). However, the idea of such a GSH-copper cotransport into the IMS or mitochondrial matrix has been challenged by experiments in yeast depleted in GSH that had wild-type

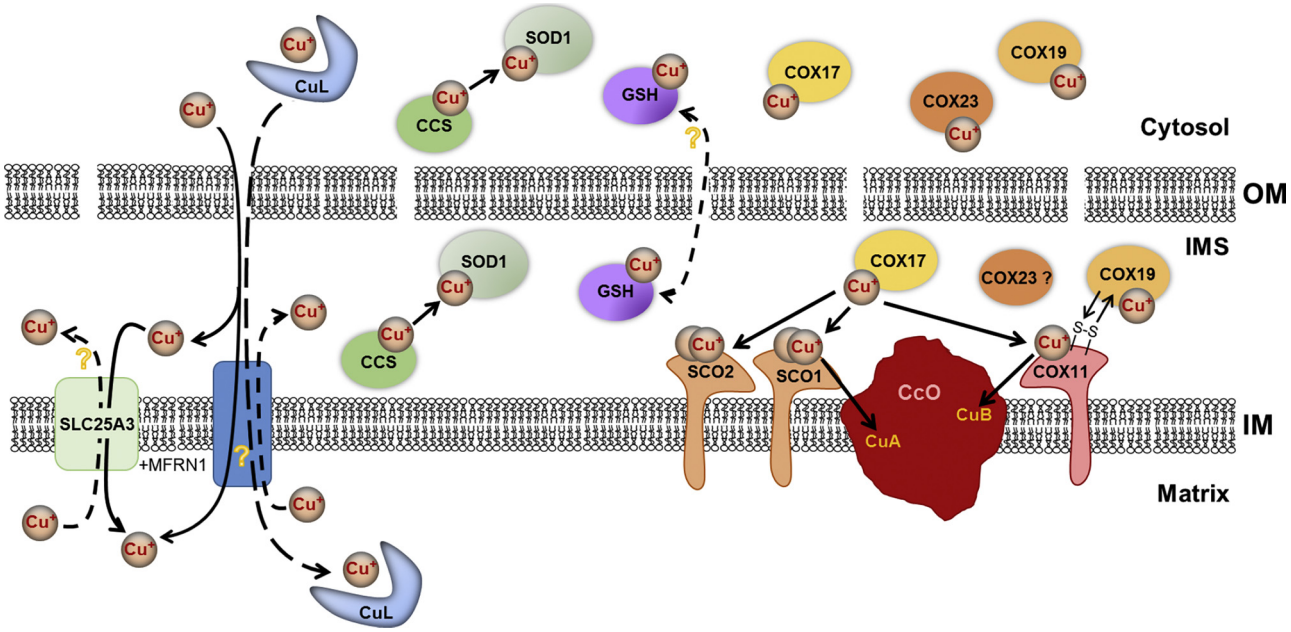

Fig. 1. Copper trafficking within the mitochondrion.
How copper enters mitochondria, either via an anionic non-protein copper ligand (CuL) or via glutathione (GSH), is currently under debate. The inner membrane proteins SLC25A3 or MFRN1 have been suggested to transport copper to the mitochondrial matrix, where it may be stored bound to CuL. In the intermembrane space (IMS), the copper chaperons COX17, SCO1 and SCO2 hand over copper by protein-protein interactions to metalize the CuA and CuB sites of the cytochrome c oxidase (CcO). The copper binding proteins COX23 and COX19 are involved in CcO assembly. COX19 has been proposed to regulate copper transfer to COX11 via modulation of its redox state. In contrast, the specific function of COX23 in copper trafficking is presently unclear. The copper chaperon CCS delivers copper to the Cu/Zn superoxide dismutase (SOD1). *CcO*, cytochrome c oxidase; *CCS*, copper chaperone for superoxide dismutase; *COX17*, cytochrome c oxidase copper chaperone 17; *COX11/19/23*, cytochrome c oxidase assembly protein 11/19/23; *CuL*, copper ligand; *GSH*, glutathione; *IM*, inner mitochondrial membrane; *IMS*, intermembrane space; *MFRN1*, mitoferrin 1; *OM*, outer mitochondrial membrane; *SCO1/2*, synthesis of cytochrome c oxidase protein 1/2; *SLC25A3*, solute carrier family 25 member 3; *SOD1*, Cu/Zn superoxide dismutase.