

Molecular mechanisms of copper homeostasis in yeast

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Abstract

Copper ions play critical roles as electron transfer intermediates in various redox reactions. The yeast *Saccharomyces cerevisiae* has served as a valuable model to study copper metabolism in eukaryotic cells. The systems for copper homeostasis; including the uptake, cytoplasmic trafficking, and metabolism in intracellular organelles, detoxification, and regulation of these systems have been characterized. Most of the molecular components for copper metabolism identified in yeast are functionally and structurally conserved in mammals. These findings have underscored the importance of evolving delicate mechanisms to utilize copper. Studies on copper metabolism in yeast certainly have opened up interesting and important research avenues that have shed light on the molecular details of copper metabolism and the physiological roles of copper.

1 Introduction

Copper (Cu) is a metal-ion abundantly found in the earth's crust. It easily accepts and donates electrons through redox reactions. Aerobic organisms have taken advantage of the chemical properties of Cu by incorporating it in various biological processes. Thus, organisms have developed mechanisms for acquiring Cu from the environment. Mechanisms for homeostatic Cu metabolism have been uncovered in prokaryotes, fungi, plants, and mammals. Among these organisms, the yeast *Saccharomyces cerevisiae* has served as a model organism to study Cu metabolism in eukaryotes. A number of experimental tools are available to understand the molecular mechanisms of Cu homeostasis. The sequencing of the yeast genome has provided an extremely valuable source of information. Deletion or expression control of yeast genes is much easier than in higher eukaryotes. Growth environments of yeast can be easily manipulated. Furthermore, most of the mechanisms and components in physiological and biochemical processes identified in yeast are conserved in higher eukaryotes.

Cu is required for at least three biological processes in yeast, (i) mitochondrial oxidative phosphorylation, (ii) superoxide anion detoxification, and (iii) iron metabolism. In the mitochondria cytochrome c oxidase subunits 1 and 2 contain Cu as an electron transport intermediate in oxidative phosphorylation (Tsukihara et al. 1995; Iwata et al. 1995). Thus, Cu is an essential micronutrient for yeast under

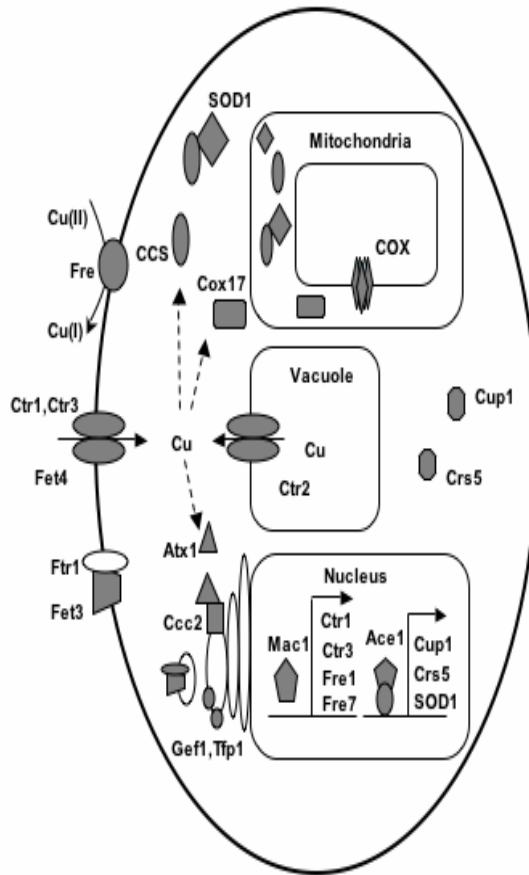


Fig. 1. Copper (Cu) homeostasis in yeast *S. cerevisiae*. Molecular mechanisms of Cu transport, distribution and detoxification have been characterized in yeast. Cu is reduced by cell surface reductases (Fre) prior to uptake by Ctr1 and Ctr3 Cu transporters. Fet4 serves as a low affinity Cu transporter. Ctr2 transports Cu from the vacuole. Cytosolic Cu chaperones Atx1, Cox17 and CCS deliver Cu to the secretory pathway, mitochondria and Cu, Zn superoxide dismutase (SOD1), respectively. At the post-Golgi vesicles Ccc2 accepts Cu from Atx1, followed by incorporation of Cu to Fet3, a multicopper ferroxidase. Gef1 and Tfp1 facilitate the transport and incorporation of Cu into Fet3. Fet3 forms a complex with the iron permease Ftr1 and both proteins are responsible for high affinity iron uptake at the plasma membrane. In mitochondria Cox17 plays essential roles in Cu incorporation into cytochrome c oxidase (COX) subunit 1 (Cox1) and subunit 2 (Cox2). CCS delivers Cu specifically to SOD1 in the cytosol. SOD1 and CCS also localize at the mitochondrial intermembrane space. Two metallothioneins, Cup1 and Crs5, are critical for Cu detoxification. Mac1 and Ace1, Cu-responsive transcription factors, regulate expression of genes involved in Cu metabolism. Mac1 and Ace1 directly bind to the *cis*-acting element of their target genes.

aerobic conditions. Yeast cells that are defective in Cu metabolism are not able to grow on media containing ethanol and glycerol as sole carbon source. These non-fermentable carbons need mitochondrial oxidative phosphorylation to generate energy and Cu serves as an essential cofactor (Keyhani and Keyhani 1975). Cu metabolism is linked to iron (Fe) metabolism, since Cu is a cofactor of Fet3 feroxi-dase, which plays an essential role in Fe transport at the plasma membrane (Dancis et al. 1994a; Askwith et al. 1994). Cu is also a critical cofactor for Cu,Zn superoxide dismutase (Cu/Zn SOD, SOD1), which detoxifies superoxide anions generated by aerobic biological processes. Thus, yeast cells defective in Cu uptake exhibit sensitivity to superoxide anion stress (Greco et al. 1990). The counterparts of these Cu-containing proteins in higher eukaryotes play the same roles as those in yeast cells (Peña et al. 1999).

Cu is able to catalyze reactions generating reactive oxygen intermediates that are highly toxic to all cellular components (Halliwell and Gutteridge 1984, 1990). The beneficial and detrimental roles of Cu in biological systems demand that cells maintain delicate control of intracellular Cu metabolism. Cu-chelating metallothionein is known as a primary defense system against Cu toxicity (Hamer 1986). Molecular characterization of the metallothionein gene and its transcription regulation has provided an initial picture that reflects the significance of homeostatic Cu metabolism. Further understanding of Cu-metabolism has been taken by cloning the genes encoding components involved in Cu uptake and intracellular distribution in yeast (Fig. 1). Phenotypic analyses of yeast cells defective in any of these components led to the identification and characterization of their counterparts in higher eukaryotes.

This chapter will address molecular mechanisms of Cu metabolism in yeast, including baker's yeast, fission yeast and pathogenic yeast, by which uptake, distribution and detoxification of Cu are precisely controlled. The major focus will be in the baker's yeast *S. cerevisiae*. Although this chapter describes the most updated information regarding yeast Cu metabolism in a comprehensive manner, there are excellent review articles focusing on specific topics in Cu metabolism such as transporters (Labbé and Thiele 1999; Puig and Thiele 2002), intracellular distribution (O'Halloran and Culotta 2000; Rosenzweig 2001; Huffman and O'Halloran 2001), mitochondrial Cu metabolism (Carr and Winge 2003), and transcriptional regulation (Thiele 1992; Winge 1998).

2 Cu uptake at the plasma membrane

2.1 High affinity Cu transporters

Initial studies on Cu uptake in yeast demonstrated that Cu transport at the plasma membrane is a saturable and carrier-mediated process (Lin and Kosman 1990). An elegant genetic approach identified the Copper Transporter 1 (Ctr1), which plays critical roles in Cu metabolism (Dancis et al. 1994a). Ctr1 was actually identified from yeast mutants that are unable to transport iron. Subsequent analysis demon-

strated that the Fe-deficiency phenotypes of the Ctr1 mutants were the consequence of Cu deficiency and that Ctr1 is a Cu transporter. Indeed, Cu transported through Ctr1 serves as a cofactor of the Fet3 ferroxidase required for Fe uptake (Askwith et al. 1994). The mammalian Cu-containing ferroxidases, ceruloplasmin and hephaestin, also play an important role in Fe metabolism in mammals (Osaki and Johnson 1969; Vulpe et al. 1999).

Ctr1 appears to be specific for Cu, since Ctr1-mediated Cu uptake is not competed by other metal-ions such as Fe^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , or Zn^{2+} (Dancis et al. 1994a). Expression of Ctr1 is a limiting factor in Cu uptake, since overexpression of Ctr1 increases Cu uptake. Non-functional mutations of the Ctr1 gene result in altered cellular responses to extracellular Cu(I), demonstrating a physiological role for Ctr1 in delivering bio-available Cu. Ctr1-defective cells exhibit deficiency in Cu/Zn SOD activities resulting in growth arrest in Cu-deficient media (Dancis et al. 1994b).

Ctr1 is a 406 amino acid integral membrane protein with three putative transmembrane domains. Ctr1 is heavily glycosylated with *O*-linkages (Dancis et al. 1994b) but it is not known whether glycosylation of Ctr1 is essential for its membrane localization and function. The hydrophilic amino terminal contains eight MXXM or MXM sequence repeats that have been identified as Cu-binding motifs in other Cu-transporting proteins from prokaryotes (Cha and Cooksey 1991; Odermatt et al. 1993). The amino-terminus of Ctr1 localizes at the extra-cellular surface of the plasma membrane (Puig et al. 2002), and those methionine residues appear to play a role in capturing Cu from the environment. Sequence alignment of Ctr family proteins has revealed that a methionine at the extracellular domain and two methionines (MXXXM) at the second transmembrane domain are conserved among these transporters. Site-directed mutagenesis experiments have demonstrated that the conserved methionines are critical for Ctr1 function (Puig et al. 2002). The methionines may serve as Cu ligands in Ctr1-mediated Cu transport. Given that most of the membrane transport proteins possess more than 6 trans-membrane domains, it is reasonable to predict that Ctr1 assembles in a homo or hetero multimer to make a Cu channel at the membrane. This is supported by *in vitro* cross-linking experiments (Dancis et al. 1994b). Interactions of glutamate residues at the third transmembrane domain of each monomer have been implicated in multimer formation (Aller et al. 2004).

Ctr3 was identified in mutant yeast cells defective in Ctr1 (Knight et al. 1996). Ctr3 is a 241 amino acid protein that has 11 cysteine residues of which three pairs are arranged in a potential CXC or CXXC metal binding motif. Ctr3 expression in many laboratory yeast strains is blocked by Ty2, a transposable DNA element. In strains that do not possess a Ty2 transposon, Ctr3 expression is regulated by Cu as is Ctr1. Ctr3 is able to replace Ctr1 function and has the same basic structural features as Ctr1, including three transmembrane domains and a functionally important MXXM motif at the second transmembrane domain. Despite these similarities, Ctr3 bears little sequence identity to Ctr1. The expression of both proteins provides enhanced proficiency in Cu uptake under Cu-limiting conditions. However, it is not clear whether Ctr1 and Ctr3 have any specificity in their roles.

Cu transporters have been identified from other yeast species as well as plants, fruit fly, and mammals due to their functional and structural similarity to yeast Ctr1 or Ctr3 Cu transporters (Puig and Thiele 2002). The structural features observed in yeast Ctr1 are common to the Ctr family of Cu transporters, which suggests that Ctr1 proteins in different organisms function with the same mode of action. However, the actual mechanisms of Ctr1 and Ctr3-mediated Cu transport are poorly understood. In mammalian cells, Fe-containing transferrins bind to their plasma membrane receptor, and Fe is released from transferrin at the endosomes to be transported by a transporter. Similarly, Ctr proteins may simply serve as Cu receptors at the cell membrane. Ctr1 endocytosis may be one mechanism for Cu transport. However, since endocytosis of Ctr3 has not been observed (Peña et al. 2000) as has been for yeast and human Ctr1, endocytosis-mediated intracellular compartmentalization of Cu transporters does not appear to be an absolutely required process in Cu acquisition in yeast. A Cu transport assay of purified Ctr1 protein in a lipid vesicle will be an approach for direct demonstration of Cu transport by Ctr1.

Given that Ctr1 does not possess an obvious ATPase domain, the driving force for Ctr1-mediated Cu transport is not known. Initial characterization of Cu transporting activities in yeast cells suggested a temperature and ATP-dependent high affinity Cu transport process (Lin and Kosman 1990). It is possible that another subunit binding to Ctr1 is an ATPase. Electrochemical measurements have shown that Cu-ion uptake is coupled with K^+ efflux in a 1:2 stoichiometry (De Rome and Gadd 1987), suggesting that Cu transport may take place via a $Cu^+/2K^+$ antiport mechanism. Since elevated extra-cellular K^+ levels enhance Cu uptake in mammalian cells (Lee et al. 2002), there may be a connection between the transport of K^+ and Cu.

An interesting observation is that the Ctr1 Cu transporter determines intracellular accumulation of cisplatin (Ishida et al. 2002). Cisplatin, a platinum-based anticancer drug, is highly active against a wide variety of tumors; however, resistance to this drug upon treatment limits its effectiveness (Loehrer and Einhorn 1984; Giaccacone 2000). A genetic screen of yeast loss-of-function mutants for cisplatin resistance has uncovered that deletion of the *CTR1* gene results in increased cisplatin resistance and reduced intracellular accumulation of cisplatin. Furthermore, cisplatin regulates Ctr1 stability and trafficking in similar ways as Cu in yeast cells. Cu pre-treatment reduces toxicity of cisplatin. These results suggest that Ctr1 transports cisplatin as well. This has been observed for both yeast and mammalian Cu transporters (Ishida et al. 2002; Lin et al. 2002). The link between Cu transporters and cisplatin may explain some cases of resistance in humans and suggest ways of modulating sensitivity and toxicity to this important anticancer drug.

2.2 Cu transporters identified from other yeast

The fission yeast *S. pombe* is particularly interesting due to its somewhat unique system of Cu uptake. A high affinity Cu uptake protein from *S. pombe*, Ctr4, re-

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