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Review

Copper homeostasis in eukaryotes: Teetering on a tightrope

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Abstract

The transition metal copper is an essential trace element for both prokaryotes and eukaryotes. However, intracellular free copper has to be strictly limited due to its toxic side effects, not least the generation of reactive oxygen species (ROS) via redox cycling. Thus, all organisms have sophisticated copper homeostasis mechanisms that regulate uptake, distribution, sequestration and export of copper. From insects to mammals, metal-responsive transcription factor (MTF-1), a zinc finger transcription factor, controls expression of metallothioneins and other components involved in heavy metal homeostasis. In the fruit fly *Drosophila*, MTF-1 paradoxically acts as an activator under both high and low copper concentrations. Namely, under high copper conditions, MTF-1 activates metallothioneins in order to protect the cell, while under low copper conditions MTF-1 activates the copper importer Ctr1B in order to acquire scarce copper from the surroundings. This review highlights the current knowledge of copper homeostasis in eukaryotes with a focus on *Drosophila* and the role of MTF-1.

Keywords: Copper scarcity; Copper importers; Ctr1B; Copper load; Metallothioneins; Copper detoxification; MTF-1

1. Biological importance of copper

Copper is an essential trace element that plays a vital role as a catalytic co-factor for a variety of metalloenzymes including superoxide dismutase (for protection against free radicals), cytochrome *c* oxidase (mitochondrial electron transport chain), tyrosinase (pigmentation), peptidylglycine alpha-amidating mono-oxygenase (PAM) (neuropeptide and peptide hormone processing) and lysyl oxidase (collagen maturation) [1–3]. At the same time, copper is toxic to both eukaryotic and prokaryotic cells, not least due to its ability to catalyze, via the so-called Fenton reaction, the generation of aggressive free radicals. Also by binding ectopically to proteins, copper can disturb their structure [4–7]. Therefore, every organism has a number of elaborate mechanisms at its disposal to control cellular uptake, distribution, detoxification and elimination of copper [8–11]. Our understanding of the systems that maintain copper homeostasis has improved considerably with the characterization of prokaryotic and eukaryotic copper transporters that mediate cellular copper uptake or efflux, as well as by the characterization of copper chaperones, a group of proteins required for binding to imported copper and delivering it to specific target proteins within the cell [1,8–13]. In this review we attempt an overview of the mechanisms that ensure copper homeostasis in various organisms, especially in the fruit fly *Drosophila*. Defects in any of these mechanisms can have dire consequences, as demonstrated by experimental manipulation in genetic model organisms and by the naturally occurring genetic defects in humans that cause severe, if not deadly diseases such as Menkes disease and Wilson disease [14–17].

2. Copper-associated diseases

Ingested metallic copper is hardly toxic due to its insolubility, and toxicosis is usually caused by contaminating traces of arsenic or lead [18]. However, inhalation of copper dust from industrial processes causes “copper fever”, a condition reminiscent to zinc fever. Initial symptoms are a sweetish taste in the mouth, dryness of the throat and a burning sensation in the eyes, followed after a few hours by strong headache, leukocytosis, general fatigue and catarrhic symptoms. If the copper source is removed, symptoms disappear within days although high copper levels remain in the blood and

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later in the urine [18–20]. Even in a soluble form such as copper sulfate, copper is relatively harmless considering its potential to wreak intracellular havoc [18]. This is due to elaborate cellular scavenging and excretion mechanisms [8–12,21]. Several human disorders result from imbalance of copper homeostasis, due to chronic copper deficiency or overload, and/or genetic predisposition [14–17,22–27]. They include Menkes disease, Wilson disease [14,22], Indian childhood cirrhosis [23], Endemic Tyrolean infantile cirrhosis (due to cow's milk contaminated with copper from untinned copper or brass vessels) [24], and idiopathic copper toxicosis (an autosomal-recessive inherited defect in copper metabolism combined with excess dietary copper) [25]. The metabolism of copper and iron is linked from yeast to mammals via copper-containing ferroxidases. For example, the genetic disease aceruloplasminemia, caused by the lack of ceruloplasmin, a multicopper ferroxidase, results in severe iron deficit and anemia [26]. In Menkes and Wilson disease the genes affected, *ATP7A* and *ATP7B*, respectively, have been characterized in great detail (see below). In dogs, it was shown that mutation of the *Murr1/COMMD1* gene is associated with copper accumulation, resulting in liver cirrhosis [27]. Intriguingly, COMMD1 interacts with the copper transporter ATP7B and also with XIAP (X-linked inhibitor of apoptosis protein), a copper-binding protein that can regulate COMMD1 levels via ubiquitination [28,29]. Independently of COMMD1, the activity of XIAP is regulated by copper binding which results in loss of caspase inhibition and thus a lowered threshold for apoptotic cell death [29].

A deficiency of bioavailable copper in the brain may contribute to the pathogenesis of neurodegenerative disorders, notably Alzheimer's disease (AD) [30,31]. Reduced activities of several cuproenzymes such as cyclooxygenase (COX), Cu, Zn-superoxide dismutase (SOD1) and peptidylglycine alpha-amidating mono-oxygenase (PAM) are observed in the brain of Alzheimer patients [32–34]. Abnormalities in copper homeostasis are also associated with spongiform encephalopathies, commonly referred to as prion diseases, and probably Parkinson's disease [31,35]. A cancer connection of copper is suggested by the recent finding that the cuproenzyme lysyl oxidase is required for hypoxia-induced metastasis [36].

3. Uptake

3.1. Copper importers in yeast and mammals

From yeast to humans, copper is acquired by high affinity, membrane-associated copper importers exemplified by the copper transporter(Ctr)-family. Copper exists in two oxidation states, namely, Cu(I) and the more stable oxidized form Cu(II). Cu(I) is the substrate for the Ctr family members which are relatively small proteins containing three transmembrane domains [37–42]. A conserved feature of some Ctr importers is an N-terminal segment that contains one or more "Mets" motifs (MxxM or MxM) which have been shown by deletion studies in yeast and human cells to be important for survival under copper starvation. These "Mets" motifs are part of the extracellular domain and are involved in the acquisition of copper ions for facilitated import [37,43,44]. There is evidence that functional Ctr importers form a trimeric complex [37,42–45]. Unlike some other high affinity metal transporters (such as ATP7, see below), Ctr proteins do not require ATP for copper import [37,46]. Their transport ability is stimulated by extracellular K^+ and probably facilitated by the extremely low intracellular concentration of free copper; while the concentration of intracellular total copper is approximately 10 to 100 micro molar, free copper, e.g., copper not bound to proteins, is several orders of magnitude lower due to an instant association of imported copper with chaperones, scavengers and other proteins [37,46,47]. In yeast, three copper transporters termed yCtr1, yCtr2 and yCtr3 have been described ([21,42,45,48], Table1). yCtr1 and yCtr3 are functionally redundant, plasma membrane-integrated, high-affinity copper transporters. Extracellular copper, usually Cu(II), has to be reduced to Cu(I) by plasma membrane reductases encoded by FRE1 and FRE2 before being imported by yCtr1 and yCtr3 [11,21,37,42,45,46]. Any excess copper is sequestered in the vacuole (somewhat analogous to the mammalian lysosome), a storage container of yeast for substances intended for recycling/degradation, including valuable metabolites such as phosphate, selected amino acids, metals, and sequestered toxins [49]. yCtr2 is localized in the vacuolar membrane and upon copper depletion, imports copper from the vacuole to the cytoplasm. Thus yCtr2 also plays an important role in yeast copper homeostasis [48]. The function of all of these copper importers was characterized by specific mutagenesis and by targeted gene disruption (see below) [21,37–40,42–46].

Complementation studies in yeast for the defective copper uptake phenotype of y*ctr1* and *yctr3* double mutant led to the identification of Ctr homologs in human [37,39]. Human cells contain two Ctr proteins, designated hCtr1 and hCtr2. hCTR1 is a 190 amino acid protein with three transmembrane domains and is the main cellular copper importer. Similar to yCtr1, the

Table 1
Genes involved in *Drosophila* copper homeostasis

Biological function	Genes (homologs in yeast/mammals)	References
Copper uptake	Ctr1A, Ctr1B, Ctr1C (yCtr1-3/hCtr1, hCtr2)	[37,40,42,51]
Copper chaperones/ trafficking	CG32446 (Atx1/Atox1) CCS (yCCS/hCCS) CG9065 (yCox17/hCox17)	[42,58,59,60,84,85]
Copper transport/ efflux	DmATP7 (Ccc2/ATP7A, ATP7B)	[10,13,37,42,74,84,85]
Copper sequestration/ detoxification	Mtn A (major role) [1] ("Cup1"/"MT-I, MT-II") [2]	[54,88,94]
Metalloregulatory protein	dMTF-1 ("Ace1", "Mac1"/MTF-1) [3]	[11,42,51,54,107,108,110]

[1] MtnB, MtnC and MtnD play minor roles in copper homeostasis.
[2] Since the primary sequence of metallothioneins is so divergent between yeast, *Drosophila* and mammals, that metallothionein genes might have evolved independently (but all with role as metal scavengers).
[3] In yeast, two transcription factors unrelated to MTF-1, designated Ace1 and Mac1, handle high and low copper, respectively.