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.

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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 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 catarrhal symptoms. If the copper source is removed, symptoms disappear within days although high copper levels remain in the blood and...
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 uninned 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 defect 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 yctr1 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

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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.
N-terminus of hCtr1 is extracellular and the C-terminus is cytoplasmic. Both hCtr1 and hCtr2 contain a conserved, C-terminal motif of cysteines and histidines termed the HCH motif [37,39,46]. Recent findings indicate that cysteine-189 of this HCH motif is important for trimerization of hCtr1 which is essential for function [50]. In this context, it is worth mentioning that hCtr1, but not its paralog hCtr2, is able to complement the defective copper uptake phenotype of the yctr1 and yctr3 double mutant. Although hCtr2 is ubiquitously expressed at the mRNA level, its function awaits to be elucidated [37,39].

3.2. Copper importers in Drosophila

*Drosophila* also contains three copper transporters of the Ctr1-family (Ctr1A, B and C), each with a specific expression pattern ([40]; Table 1). Ctr1A is constitutively expressed at all stages, from four-hour-old embryos to adults and also in cultured S2 cells from *Drosophila*. Ctr1B is abundantly expressed in late embryonic stages and in larvae, but less in early embryonic stages and adults. Unlike Ctr1A, Ctr1B and Ctr1C are not detectable in S2 cell culture. Ctr1C expression is confined to late larval stages and, interestingly, to adult males. These expression patterns suggest that Ctr1 genes have distinct roles in copper homeostasis in *Drosophila* [40]. By analogy to yeast copper importers (see below), Ctr proteins in *Drosophila* may localize to different cell compartments. So far, it is known that in intestinal cells Ctr1B is localized to the apical side of the plasma membrane, where it is responsible for copper uptake in the larval gut ([40,42,51]; D. Egli, K.B. and W.S., unpublished data). The Ctr1B gene is transcriptionally activated upon copper starvation [40], and it was shown that this transporter is regulated via the same transcription factor (MTF-1) that otherwise regulates the activation of metal scavengers upon copper load [51] (see also below). Besides an uptake via specific Ctr importers, copper can also be imported by less specific general metal transporters [37,42]. However, as shown in yeast and *Drosophila*, these transporters can only ensure a sufficient copper supply when dietary copper is abundant, and thus are unable to substitute for a loss of Ctrs [12,21,37–40,42,46,49,51].

3.3. Lessons from deletion and overexpression of copper importers

Menkes disease not withstanding, which is characterized by severe copper deficiency in most organs due to malabsorption, there are no known hereditary diseases with symptoms resembling copper starvation. However, genetically manipulated model organisms have provided ample evidence for the important role of copper homeostasis. In yeast, elimination of yCtr1 copper transporter causes defects both in copper uptake and, indirectly, in iron uptake, due to the fact the copper-dependent ferroxidase Fet3p is essential for iron uptake [21,42,52]. Ctr1 deletion by itself does not lead to intracellular copper deprivation, but deletion of both yCtr1 and yCtr3 does [1,21,42,52,53]. In higher eukaryotes, the most detailed studies were performed in *Drosophila*. The phenotype of *Ctr1B* mutants was akin to that of a deletion of the regulatory protein MTF-1: *Ctr1B* mutants are particularly sensitive to copper scarcity and, to a lesser extent, also to copper load [40,51,54]. The opposite genetic condition, namely, overexpression of Ctr1B, resulted in a hypersensitivity to copper such that the flies were not viable when grown on food of normal copper content [51]. Interestingly, this latter phenotype was rescued by dietary copper starvation. Expression of Ctr1B under the control of the eye-specific promoter (GMR) resulted in aberrantly developed eyes (“rough eye” phenotype) and this phenotype was aggravated by copper supplementation in a dose-dependent manner. Again, limiting dietary copper rescued the developmental toxicity phenotype (H. Hua and W.S., unpublished data). A rescue effect was also seen upon overexpression of proteins with a role in metal detoxification (metallothioneins, MTF-1, DmATP7; see below) (D. Egli, K.B. and W.S., unpublished data). Knockout mice for the copper transporter Ctr invariably die at mid-gestation, underlining the essential function of Ctr1 in copper acquisition during mammalian embryonic development [55,56]. Unlike the congenital zinc deficiency disorder Acrodermatitis enteropathica, which can readily be treated by extra doses of dietary zinc, the Ctr knockout cannot be rescued by extra copper supply to the pregnant female [55–57].

4. Distribution

4.1. Delivery of copper: copper chaperones

In contrast to zinc, which is required as a structural or catalytic co-factor in hundreds of proteins, copper is part of a considerably lower number of proteins. This fact, combined with the extreme toxicity of free intracellular copper, is probably the reason why organisms from prokaryotes to mammals are in possession of specific copper chaperones that bind copper with high affinity and deliver it to specific target proteins. They are probably important also to compete with the non-specific copper binding sites of many cellular proteins in various subcellular compartments. From yeast to humans, three functional groups of chaperones are conserved ([58–60], Table 1). Atx1 is a cytosolic yeast chaperone that delivers copper to the transport ATPase Ccc2 in the trans-Golgi network. From there, Ccc2 translocates copper into vesicles where it is loaded into the multicopper containing protein ferroxidase Fet3 [61]. The human homolog of Atx1, Atox1, delivers copper to Menkes and Wilson disease ATPase transporters ATP7A (or MND) and ATP7B (or WND), respectively. While ATP7A delivers copper to copper-dependent enzymes in the secretory pathway, ATP7B directs copper incorporation into ceruloplasmin, a serum ferroxidase that contains more than 95% of the copper found in blood plasma ([62,63], see also Section 4.2). ATP7A, also called Menkes disease protein, directs copper within the trans-Golgi network to several cupro-enzymes including tyrosinase ([10,13,37]; Fig. 1). A bacterial homolog of Atox1 called CopZ, has also been identified [64]. The copper chaperones for superoxide dismutase, referred to as CCS proteins, donate copper to the eukaryotic antioxidant enzyme Cu,Zn-superoxide dismutase (SOD1) [65]. Mice with a targeted disruption of CCS exhibit marked reductions in
SOD1 activity [66]. In the case of CCS, the copper delivery mechanism has been elucidated to a large extent. CCS has a structure that resembles the copper binding domain of SOD and mediates the formation of a transient heterodimer between CCS and SOD. This heterodimer resembles the SOD homodimer and facilitates copper transfer to SOD [67]. Three proteins, Cox17, Cox11 and Sco1, play a role in the assembly of mitochondrial cytochrome c oxidase [68]. Cox17 is proposed to deliver copper to the inner mitochondrial membrane proteins Cox11 and Sco1, which in turn may transfer it to cytochrome c oxidase (Cco) ([42,68,69], see also Fig. 1). Some fungi such as Podospora anserina are able to maintain respiration even upon insufficient copper supply, switching from COX to AOX, an iron-containing alternative oxidase [70]. From yeast to humans, several studies have shown that Cu(I) forms a binuclear cluster (cuprous-thiolate) intermediate with the cysteine residues of chaperones such as yCox17 and hCCS [71–73].

4.2. Copper-transporting P-type ATPases

Unlike yeast, multicellular organisms have the additional problem that copper not only has to be taken up by cells of the digestive tract, but that there also has to be an adequate copper
supply to remote organs. For this task, they rely heavily on the aforementioned ATP-dependent transporters of the ATP7 family. These heavy-metal-transporting P-type ATPases are conserved from bacteria to mammals ([10–13,74,75], Table 1), whereby the latter contain two isoforms termed ATP7A and ATP7B. ATP7A is expressed in the intestinal epithelium as well as most other tissues other than liver. Both ATP7A and ATP7B undergo copper-stimulated trafficking [10,13,14,76–78]. At elevated copper levels, ATP7A, i.e., Menkes disease protein moves from the trans-Golgi network to the plasma membrane and redistributes excess copper by pumping out from the cell. ATP7A returns to the trans-Golgi network as soon as the intracellular copper levels are reduced. ATP7A, which is also expressed in mucosal cells lining the intestine, is pivotal for systemic absorption of copper in these cells as well as reabsorption in the kidneys [10,13,22,77,79]. ATP7A is particularly important for adequate copper supply to the brain and any impairment in the activity of this protein leads to the very severe Menkes disease, an X-linked recessive disorder that presents with symptoms including stunted growth, skeletal defects and progressive degeneration of the central nervous system, often fatal during early childhood [10,14,22,77].

Vertebrates including humans possess ATP7B, a liver-specific copper transporter that also plays a central role in the transport of copper across cellular membranes [10,13,14,74]. ATP7B is normally expressed in liver and kidney and to a lesser extent in the brain. It is structurally and functionally related to ATP7A and the two corresponding genes must have arisen by duplication [80]. Normally ATP7B, like ATP7A in other tissues, is involved in transporting copper into the secretory pathway for incorporation into apoceruloplasmin in the trans-Golgi network. At high copper load, ATP7B trafficks to the biliary canalicular membrane where excess copper is excreted into the bile duct for disposal from the body via digestive tract [10,13,22,74,79,81,82]. Loss-of-function mutations in the ATP7B gene leads to Wilson disease, an autosomal recessive disease which causes accumulation of copper in the liver and a life-threatening liver cirrhosis due to a deficiency of biliary copper secretion [10,13,22,74,82]. Besides liver cirrhosis, Wilson disease can also present with neurological symptoms such as behavioral disturbances and movement disorders [22,82,83].

Drosophila has a sole ortholog of mammalian Menkes and Wilson genes, DmATP7, which is essential for in vivo copper distribution. Recent studies have shown that DmATP7 plays at least two roles at the cellular level: i) delivering copper to cuproenzymes required for pigmentation and neuronal function; and ii) removing excess cellular copper via facilitated efflux [84,85]. Similar to Menkes disease, the loss of this ATPase gene in Drosophila results in hyperaccumulation of copper in intestinal cells but copper starvation in the rest of the body [85].

5. Detoxification/elimination

5.1. Metallothioneins: a safeguard of the cell

Even though copper export and import are subject to elaborate control mechanisms, there can be conditions where copper is imported in excess. For example, Drosophila Ctr1B, the major larval copper importer, is regulated at the transcriptional level while in other species the Ctrs are regulated posttranscriptionally. However, both downregulation mechanisms may not be fast enough to cope with a sudden increase in ambient copper concentration ([51,86], D. Egli, K.B. and W.S., unpublished data). Therefore, an important aspect of metal homeostasis is the sequestration of toxic heavy metals, especially copper, a task that is mainly performed by the metallothioneins. Metallothioneins constitute a group of low molecular weight, cysteine-rich proteins with outstanding metal binding capacity [87,88]. They are found in all eukaryotes as well as in some prokaryotes and mainly function as metal scavengers/metal storage proteins ([54,89–92]; Table 1). Typically metallothionein genes are expressed at a basal level but their transcription is strongly induced upon heavy metal load [54,91,92]. Even though metallothioneins are the least conserved of the proteins described in this review (importers, exporters, chaperones, transcription factors, scavengers), they share the common feature of a very high proportion of cysteines which enables them to avidly bind toxic metals while also playing a role in cellular redox balance and radical scavenging. Budding yeast Saccharomyces cerevisiae contains two metallothioneins, Cup1 and CRS5 [93,94]. These are copper-thioneins and their inactivation leads to copper sensitivity, while their overexpression confers resistance to copper toxicity [93–95]. Of the two, Cup1 seems to play a dominant role in neutralizing excess intracellular copper [94]. In Drosophila, there are four metallothioneins, namely, MtnA, MtnB, MtnC and MtnD [54]. MtnA is primarily expressed during embryogenesis, while MtnB is found in late stage embryos, larvae, and in adult flies within the gut, Malpighian tubules, fat body and hemocytes [96–98]. MtnC and MtnD have arisen most likely by duplications from the MtnB gene and have a lesser role in protecting against metal toxicity [54,99]. Drosophila mutants with a disruption of all four metallothionein genes (“family knockout”) are highly sensitive to elevated copper concentrations [99]. Interestingly, metallothionein expression and the sites of copper accumulation coincide, especially in the cytoplasm of so-called copper cells of the midgut and also in the posterior midgut [100,101]. These copper cells, or cuprophilic cells, upon copper load display a peculiar orange copper luminescence caused by a copper–metallothionein complex. This copper luminescence rapidly disappears if larvae are transferred to copper-depleted food, presumably because copper is provided to the growing organism [99,102].

In vertebrates, the metallothioneins are small proteins but still larger than those of Drosophila and yeast. Among the approximately 60 amino acids of a typical vertebrate metallothionein, close to one third are cysteines. All mammals contain four major members of the metallothionein family, termed MT-I to MT-IV (or MT-1 to MT-4) [87,88]. In the mouse, each type is represented by one member, while in humans, MT-I has expanded into a gene family of its own. MT-I and MT-II are expressed at all stages of development in most, if not all cell types. Their transcription is responsive to adverse conditions, especially heavy metal load, oxidative stress,
ionizing radiation and a number of other stress conditions [87,88,91,103]. MT-III is constitutively expressed, predominantly in neurons but also in glia and male reproductive organs, and its elimination in the mouse increases susceptibility to seizures [104]. MT-IV expression is confined to differentiated squamous epithelia [105]. The MT-IV gene, which also contains a metal response element (MRE) in the promoter region, is at least partially metal responsive [105].

5.2. Metallothioneins and ATPase transporters handle excess cellular copper

Metallothioneins share a high cysteine content and the property to avidly bind heavy metals but otherwise can be quite divergent in their primary sequence. Nevertheless, vertebrate metallothioneins from fish to humans display sequence similarity, which suggests that apart from sequestering heavy metals, they also play additional role(s) by interacting with specific cellular proteins [87,88,91,103,106,107]. While the role of metallothioneins in metal detoxification is established beyond doubt, not least by the complete knockout of all four Drosophila family members [99,102], metallothioneins probably serve as a metal source during a transient metal scarcity. However, a detailed analysis of Drosophila knockout flies has not provided any evidence that a lack of metallothioneins would reduce fitness under chronic copper starvation. In line with the latter finding, metallothionein expression is strongly diminished in wild type flies under limiting copper conditions [51,99].

Metallothioneins can store copper but they are not suitable to eliminate an excess of it from the cell. For this, there are the aforementioned ATP7 transporters, which in some multicellular organisms redistribute copper from one cell to another but do not affect total body copper. In vertebrates, as mentioned before, there is nevertheless a specific way of eliminating excess copper from the organism utilizing the liver-specific ATP7B Wilson transporter, which upon copper load translocates from the Golgi to the plasma membrane to export copper into the so-called canaliculi, the smallest bile transport channels that ultimately lead into the gall bladder [10,13,74,77,79,82].

6. Transcriptional regulation of copper homeostasis: from yeast to mammals

As mentioned, the expression of metallothioneins is regulated at the transcriptional level. Upon heavy metal load, specific metal-responsive transcription factors bind to metallo-thionein gene promoters to boost their expression. In the budding yeast S. cerevisiae, the responsible factor is Ace1. The amino-terminal half of Ace1, which is rich in cysteines and

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**Fig. 2.** Overview of the transcription factors regulating copper homeostasis in different organisms. In the yeast *Saccharomyces cerevisiae*, two copper-regulated proteins are involved in maintaining copper levels: Mac1 at low copper and Ace1 at high copper. In *Drosophila*, the response to both copper load and copper scarcity is handled solely via dMTF-1, the homolog of mammalian MTF-1 (metal-responsive transcription factor-1). In humans, copper importers are regulated posttranslationally; therefore, copper-regulated transcription factors may not be involved (see text).
positively charged amino acids, harbors a copper-dependent DNA binding domain. The carboxyl-terminal half is highly acidic in nature and mediates contacts to the transcription apparatus [108,109]. In the yeast metallothionein gene promoters, Ace1 binds to cis-regulatory elements (UAS$_{Cu}$, core consensus sequence 5′-GCTG-3′) that mediate transcriptional activation by copper [108,109]. For the converse situation of copper starvation, yeast relies on Mac1, a distinct transcription factor. Mac1 is also regulated by copper, whereby the copper-binding domain acts as an intramolecular auto-inhibitory domain [110–112]. Curiously, in the fission yeast Schizosaccharomyces pombe, Cuf1, a factor more closely related to S. cerevisiae Ace1 than to Mac1, is activated by copper starvation [113]. From insects to mammals, the main transcription regulator for handling heavy metal load, including copper excess, is MTF-1 (metal-responsive element binding transcription factor-1 or metal-responsive transcription factor-1) (see below) (Table 1 and Fig. 2) [54,107,114–117].

### 6.1. MTF-1 in mammals and Drosophila

MTF-1 and its cognate binding site, metal-response element (MRE, core consensus TGCRNC) of higher eukaryotes is the functional analog of yeast Ace1, but neither the two factors nor their binding sites are related to each other [107–112]. The DNA binding domain of MTF-1 consists of six zinc fingers of the C2H2 type. Several studies have shown that MTF-1 requires a higher zinc concentration than other factors for DNA binding in vitro, which suggested a simple mode of activation by metal load [118,119]. Indeed, in a cell-free transcription system, it was shown that zinc activates human MTF-1 directly, whereas copper, cadmium, and hydrogen peroxide activate transcription indirectly by displacing zinc from zinc-saturated metallothioneins and probably also from other zinc binding cellular proteins [118,119]. However, this in vitro system does not reproduce the full complexity of the in vivo regulation of MTF-1, which also involves phosphorylation/dephosphorylation and nucleocytoplasmic shuttling [120,121]. Human MTF-1 contains an extended non-canonical nuclear localization signal (NLS) sequence that overlaps with the zinc finger domain (M. Cramer, M. Meuli, O. Georgiev and W.S., unpublished). The C-terminal part of MTF-1 downstream of the zinc fingers harbors three different transactivation domains, namely, an acidic, a proline-rich, and a domain rich in serine and threonine [107,122]. MTF-1 also contains a conserved nuclear export signal (NES) sequence (LCLSDDLSSL) overlapping with the major activation domain [120]. In non-dividing, non-stressed cells, the majority of MTF-1 is located in the cytoplasm. Upon heavy metal load, MTF-1 accumulates in the nucleus and activates transcription of its target genes via binding to MREs in the promoters [107,120]. It has been shown that the NES is essential for metal activation of human MTF-1 (M. Cramer and W.S., unpublished). Also, metal induction is blocked by broad range inhibitors of protein kinase C, which indirectly cause elevated phosphorylation of MTF-1. This suggests that specific dephosphorylation of MTF-1 might contribute to its activation [121]. Both human and Drosophila MTF-1 are highly conserved in the DNA binding zinc finger region but vary considerably outside of it [116,123]. Complementation studies have shown that both the human and Drosophila MTF-1 are partially metal responsive when introduced into flies and mammalian cells devoid of their endogenous MTF-1, respectively [123].

### 6.2. Paradoxical activity of dMTF-1

MTF-1 is a unique transcription factor in that it is able to handle both extremes, namely, copper load and copper starvation. At high copper it activates metallothionein genes, while at low copper it activates the gene for the copper importer Ctr1B. The upstream region of the Ctr1B gene, which mediates transcriptional induction upon copper scarcity, includes a segment with three uniquely spaced, strong MREs that is highly conserved among several Drosophila species. These MREs are bound by MTF-1 both at low and high copper, and due to their specific arrangement, possibly in conjunction with auxiliary factors, confer activity upon copper starvation [51] (Figs. 1 and 2).

While Drosophila Ctr1B is predominantly, if not exclusively, regulated at the transcriptional level ([51]; D. Egli, K.B. and W.S., unpublished data), yeast and human Ctrs are regulated posttranscriptionally, whereby excess copper was reported to stimulate rapid protein degradation and/or endocytosis [37,43,53,86]. Each of these mechanisms, whether transcriptional or posttranscriptional, allows for a fine tuning of Ctr levels and thus of copper import.

### 7. Conclusions

The data presented in this review emphasize the vital importance of copper homeostasis for all eukaryotes. While studies in yeast have paved the way for similar studies in multicellular organisms, important insights were also obtained from human copper-associated diseases and their mouse models. Copper homeostasis in the fruit fly Drosophila has only recently been studied intensely but the results have already contributed significantly to the field. In spite of considerable insights into the mechanisms regulating copper levels obtained in recent years, our understanding is far from complete. In Drosophila, for example, it is not clear whether there are copper-responsive repressor(s) and/or coactivator(s) of the transcription regulator MTF-1. Another question concerns the specificity of the metal response. For example, why is the Ctr1B copper importer only activated upon copper starvation, the zinc exporter Znt-35C only by excess zinc, but the metallothionein genes by most heavy metals? What is the overlap, if any, in the roles of the three copper importers Ctr1A, Ctr1B and Ctr1C? In humans, major questions concern the involvement of copper in neurodegenerative diseases such as Alzheimer, Parkinson and prion diseases. Answers to these questions will be of great medical importance and will have to come from a combination of clinical research and work with model organisms. The latter
References


