The taste of heavy metals: Gene regulation by MTF-1

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ABSTRACT

The metal-responsive transcription factor-1 (MTF-1, also termed MRE-binding transcription factor-1 or metal regulatory transcription factor-1) is a pluripotent transcriptional regulator involved in cellular adaptation to various stress conditions, primarily exposure to heavy metals but also to hypoxia or oxidative stress. MTF-1 is evolutionarily conserved from insects to humans and is the main activator of metallothionein genes, which encode small cysteine-rich proteins that can scavenge toxic heavy metals and free radicals. MTF-1 has been suggested to act as an intracellular metal sensor but evidence for direct metal sensing was scarce. Here we review recent advances in our understanding of MTF-1 regulation with a focus on the mechanism underlying heavy metal responsiveness and transcriptional activation mediated by mammalian or Drosophila MTF-1. This article is part of a Special Issue entitled: Cell Biology of Metals.

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1. Introduction

The term heavy metal comprises a number of essential and non-essential metals. Among the latter cadmium, mercury and lead are toxic even in trace amounts. Although zinc and copper, which are essential heavy metals, are integral parts of proteins, notably enzymes and transcription factors, an excess of these metals is also toxic. Therefore, elaborate systems to import, sequester, store, transport and expel metals have evolved. Important players in metal homeostasis are the metallothioneins. These small proteins with a high content of cysteines can bind and thereby sequester heavy metals. Metallothioneins were discovered in 1957 by Margoshes and Vallee as cadmium-binding proteins in preparations of horse kidney [1]. Transcription of metallothionein genes is upregulated in response to different stimuli, especially heavy metals, but also oxidative stress or stress hormones (glucocorticoids). A hallmark of the promoters/enhancers of most metallothionein genes are short DNA sequence motifs termed metal response elements (MREs); many also harbor antioxidant response elements (AREs) and glucocorticoid response elements (GREs) [2–4]. MREs were originally discovered as conspicuous DNA sequence motifs present in multiple copies in the promoter region of the mouse metallothionein 1 gene. In transfection experiments, a reporter gene driven by a synthetic, MRE-containing promoter became responsive to zinc and cadmium [5,6]. MREs, often in multiple copies, are typically present in the upstream regulatory sequences of other heavy metal-responsive genes. The identification of these MREs, that share the core consensus sequence TGCRCNC (R=A or G, N=any nucleotide), suggested the existence of a specific transcription factor that regulates metallothionein expression in response to metals [7]. Indeed in 1988 a MRE-binding protein was identified by electrophoretic mobility shift and methylation interference studies [8,9]. It is bound to its cognate DNA motif in a zinc dependent manner [8] and was termed MTF-1 (for MRE-binding transcription factor-1) [7]. In 1993 the cDNA of mouse MTF-1 was cloned which revealed it as a zinc finger protein [10]. Human MTF-1 with a length of 753 amino acids was cloned soon thereafter and found to be highly similar but slightly longer at the C-terminus than mouse MTF-1 [11,12]. A second MRE-binding protein unrelated to MTF-1, termed MT2 or ZiRF1, was found in a yeast one-hybrid assay [13]. Its significance, if any, for metal homeostasis remains unclear. Several earlier reviews have addressed aspects of MTF-1 function [14–18]. Here we attempt a presentation of the current state of knowledge incorporating new data that has arisen in the meantime.

MTF-1 is evolutionarily conserved from insects to mammals, it so far has been characterized in human, mouse, capybara, fish and Drosophila [10,11,19–24], where it is typically present as a single copy gene. Database searches also revealed orthologs of MTF-1 in the genomes of other vertebrates and insects. Genes with an array of zinc fingers typical for MTF-1 can even be tracked down in invertebrates such as sea urchin, sea anemone and hydra (K. Steiner and W.S.), but their role in metal homeostasis has not been addressed experimentally so far. While metallothionein genes are also present in C. elegans and in yeast, no MTF-1 ortholog has been identified in these organisms. In yeast metal-responsive transcription factors unrelated to MTF-1 were identified [25]. In C. elegans the GATA-type
transcription factor ELT-2 is essential for metallothionein transcription, but data about metal-sensing regulators are still missing [26].

MTF-1 not only plays a central role in the homeostasis and detoxification of heavy metals, but also helps to protect the organism against hypoxia and oxidative stress [27–32]. Furthermore, in mammals MTF-1 is essential for embryonic liver development, and mice lacking MTF-1 die at embryonic stage due to liver degeneration [33,34]. In contrast to this, Drosophila lacking MTF-1 are viable under normal conditions but are markedly sensitive to heavy metal stress [35].

Considerable efforts have been undertaken to understand how the metal-dependent activity of MTF-1 is regulated. In a subset of studies, transcript and protein levels of MTF-1 were reported to increase upon metal treatment [36–38]. However, MTF-1 under the control of a constitutive promoter is still fully functional, suggesting that its function is mainly regulated post-translationally [39]. Indeed MTF-1’s activity can be regulated at several steps: nuclear–cytoplasmic shuttling, DNA-binding, and the interaction with other transcriptional coactivators. Under normal conditions mammalian MTF-1 localizes both to the nucleus and the cytosol but accumulates in the nucleus upon diverse stresses (Fig. 1), including heavy metal overload, heat shock and oxidative stress [40,41]. Zinc sensing by MTF-1 can be mediated by the zinc fingers and a metal-responsive activation domain (see “Functional domains of MTF-1”), whereas response to other stresses is most likely indirect, via zinc released from metallothioneins and other proteins (Fig. 1). After DNA-binding MTF-1 recruits different co-activators and often relies on other transcription factors for coordinated target gene expression (see “Interaction partners of MTF-1” and Table 2).

2. Functional domains of MTF-1: an unexpected human superiority

Fig. 2A shows an overview of the domain composition of human MTF-1. Its DNA-binding domain is composed of six zinc fingers of the Cys2His2-type. Since MTF-1 shows an increased DNA-binding upon zinc supplementation [8,42] and individual zinc fingers have different zinc binding affinities [43,44], this domain was suggested to mediate the intrinsic zinc sensing of MTF-1. Zinc fingers 5 and 6 can contribute to specificity but are dispensable for MRE-binding per se, as suggested by alkylation of individual zinc fingers with NEM, proteolysis and deletion experiments [17,45,46]. Domain exchange experiments between MTF-1 and another zinc finger transcription factor (Sp1) suggested a role for zinc finger 1 of MTF-1 in zinc-dependent DNA binding [47]. Since no crystal structure of the protein/DNA complex is available, interactions between the zinc finger residues and MRE bases are extrapolated from other zinc finger/DNA complexes [17].

Some of the “linker” sequences between the zinc fingers of MTF-1 do not conform to the linker consensus sequence TGE[K/R]P which is found in approximately 40% of all multi-Cys2His2 zinc finger domains [48]. Mutation of the non-conventional linker between zinc fingers 1 and 2 of mouse MTF-1 led to permanent nuclear localization, DNA-binding and constitutive transcriptional activity [49]; the same mutation in human MTF-1 caused constitutive nuclear localization, but DNA binding and transcriptional activity remained zinc-responsive (UL and WS, unpublished). This and other results (discussed below) indicate that the mechanism of metal inducibility of human MTF-1 is more elaborate and/or more robust than the one of the mouse.

![Fig. 1. Overview of mammalian MTF-1 regulation. Under normal conditions MTF-1 is shuttling between the cytoplasm and the nucleus, with export most likely mediated by interaction of MTF-1 with Crm1 [52], which mediates nuclear export of proteins that carry a leucine-rich nuclear export signal. MTF-1 can be activated directly by zinc or indirectly by release of zinc from metallothioneins upon cadmium load or oxidative stress (H2O2), as suggested by cell free transcription experiments [123]. In addition, activity of MTF-1 can be modulated by phosphorylation. Upon zinc binding MTF-1 shuttles to the nucleus where it binds to its cognate DNA motif, termed MRE (metal-response element) with the consensus sequence TGCRCNC (R = A or G; N = any nucleotide). MTF-1 interacts with other transcription factors (e.g. Sp1) and coactivators (e.g. p300) to drive target gene expression.](image-url)
Transcriptional activation domains were functionally classified according to their amino acid composition [50]. By fusing segments of mouse MTF-1 to the Gal4 DNA-binding domain, three transcriptional activation domains were identified in the C-terminal half: an acidic, a proline-rich and a serine/threonine-rich domain [51]. The acidic activation domain is the strongest and harbors a major determinant of metal inducibility: when fused to a heterologous DNA-binding domain this chimeric transcription factor shows a relatively strong inducibility by zinc, though still not quite as high as wild type human MTF-1. However, the response via this domain was only seen in three of six cell lines investigated and thus is not as robust as that of complete human MTF-1, which is metal responsive in all mammalian cell types tested so far [52].

The subcellular distribution of human MTF-1 is regulated by distinct motifs: a non-conventional nuclear localization signal (NLS) and a nuclear export signal (NES) [40,52]. The former overlaps with zinc fingers 1–3, the latter is embedded in the acidic activation domain and resembles classical, leucine-rich NESs that mediate the interaction with the export receptor Crm1. In accordance with this notion, treatment of cells with leptomycin B (LMB), an established inhibitor of Crm1, leads to nuclear accumulation of MTF-1 [52]. These findings support the concept of MTF-1 as a nuclear-cytoplasmic shuttling protein with balanced import and export in non-stressed cells. The nuclear-cytoplasmic distribution of mouse MTF-1 was found to be coupled to activity (see above). By contrast, the significance of the NES for human MTF-1 function is not clear. Inhibition of export by LMB-treatment only slightly affected zinc-induced reporter transcript levels, again this points to a more robust metal responsiveness of human MTF-1 compared to mouse MTF-1 [52]. The NES sequence is not only required for export from the nucleus but also is an essential part of the acidic activation domain in human MTF-1. This suggests a model in which the acidic activation domain provides mutually exclusive binding to either the export receptor or a transcriptional cofactor.

In reporter assays, human MTF-1 exhibits a much stronger metal response than mouse MTF-1, irrespective of whether tested in human or mouse cells [53]. The low metal inducibility of mouse MTF-1 seems to be characteristic for rodents, since MTF-1 from capybara, the largest living rodent, behaves like mouse MTF-1 [24]. A sequence comparison revealed that the extended form of MTF-1 is the primordial form that is typical for all mammals except rodents. The proteins of mouse and capybara became truncated at the C-terminus by premature termination mutations (Fig. 2A). However, unexpectedly, the critical determinant for the different metal inducibility of rodent and human MTF-1 turned out to be elsewhere: in contrast to its human counterpart (see above) the isolated acidic activation domain of mouse MTF-1 does not show metal responsiveness. However, mouse MTF-1 can readily be “humanized” by changing three amino acids in the NES/acidic activation domain in mouse MTF-1. Such an MTF-1, despite being still truncated by 78 amino acids at the C-terminus, displays the high metal inducibility characteristic for human MTF-1. Conversely, “mousifying” the human NES decreases the metal response, showing that the NES sequence is not only required for export from the nucleus but also is an essential part of the acidic activation domain in human MTF-1 [52]. The low metal response of rodent MTF-1 relative to human MTF-1 is also observed in transfected rodent cells and on rodent metallothionein reporter constructs, i.e., it is independent of the host cell or reporter system used, and we have speculated that heavy metal stress may have been less of an issue during rodent evolution [24]. While we cannot exclude that an unknown factor(s) contributes to metal homeostasis in rodents, it is clear that the metal response of human MTF-1 is intrinsically more robust. The fact that humans have at least 10 metallothionein genes (plus a few pseudogenes) versus 4 in the mouse is consistent with the idea that long-lived organisms are particularly vulnerable to heavy metal accumulation and toxicity.

The C-terminal segment of vertebrate MTF-1 harbors a conserved cysteine-rich motif (CQCCAC) that was shown to be necessary for
metal-induced transcription [54,55]. Studies in our lab revealed that this cysteine cluster mediates homodimerization of MTF-1. Most likely only one partner of dimeric MTF-1 binds to DNA, as no conserved spacing between directly or divergently oriented MREs is present in the promoter and enhancer regions of different MTF-1 target genes (K. Steiner and W.S.). The dimerized molecule might offer a platform for the recruitment of transcriptional cofactors (Fig. 1). Although it was tempting to speculate that the cysteines are involved in metal-sensing, i.e., that homodimerization is regulated by metal binding, our data showed that dimerization is constitutive [56]. Taken together, the most recent studies bring the acidic activation domain into focus as a major mediator of metal responsiveness of human MTF-1, with homodimerization being a prerequisite.

Recently a sumoylation site at position Lys627 and a SUMO-interacting motif \( ^{627}\text{VPVIII}_{626} \) was identified for mouse MTF-1. These are conserved among vertebrates. The sumoylated form of MTF-1 resides in the cytoplasm, where it probably forms SUMO-dependent complexes [57].

_Drosophila_ also contain a MTF-1 homolog. Besides the 791 amino acid form which was cloned and characterized [21] gene annotation programs also predict an alternative _Drosophila_ MTF-1 splice form encoding a 1006 amino acid protein, which is conserved among drosophilids. In addition, on the complementary strand an open reading frame that encodes a MADF domain containing protein is present. However, both the 1006 amino acid variant of MTF-1 and the open reading frame for the putative MADF DNA-binding protein cannot be essential: in a genetic background that precludes their expression and solely relies on a cDNA encoding the standard 791 amino acid form of MTF-1, all investigated phenotypes were completely rescued.

_Drosophila_ MTF-1 is composed of domains with similar functions as those found in human MTF-1, but their primary sequence and location within the protein sometimes differ (Fig. 2B). The zinc finger region is the only domain with clear sequence homology to human MTF-1 (81% similarity, 68% identity). A nuclear localization function is harbored within zinc fingers 1–3 [58]. Mammalian and _Drosophila_ MTF-1 bind to the same core MRE consensus (Fig. 3). There is little if any protein sequence similarity outside of the zinc fingers, but nevertheless mammalian and _Drosophila_ MTF-1 can largely cross-complement each other when tested in the respective knock-out background [16].

In contrast to human MTF-1, _Drosophila_ MTF-1 contains an activation domain that is not metal-responsive but shows strong constitutive activity when fused to a heterologous DNA-binding domain. Two domains of _Drosophila_ MTF-1 seem to be involved in metal sensing: a cysteine cluster “CCTCCTC” [59] and a C-terminal cysteine-rich “metallothionein-like” domain [58]. As for its mammalian ortholog (see below) regulation of _Drosophila_ MTF-1 activity is mediated in part via phosphorylation. Four phosphorylation sites, located in the zinc finger region, were identified in a phosphopeptide screen carried out with _Drosophila_ cells [60]. Mutation of these sites strongly affected the expression of metallothionein B but not of metallothionein A, indicating a target gene discrimination even between members of the metallothionein gene family [58], discussed also below [85].

Furthermore, the C-terminal domain plays an essential role in the regulation of _Drosophila_ MTF-1. First, it autorepresses MTF-1’s transcriptional activity, likely via inhibiting DNA-binding, and second it mediates degradation of MTF-1 after prolonged heavy-metal exposure, presumably to counteract over activation of target genes [58]. The importance of the C-terminus in the regulation of MTF-1 becomes obvious when C-terminal truncations of MTF-1 are introduced into the _Drosophila_ genome: such transgenic flies suffer from female sterility, a markedly reduced lifespan and a distorted wing development, most likely as a result of excessive expression of target genes [58].

### Fig. 3. Consensus sequence of human and _Drosophila_ metal response elements. Analysis of MREs located in the promoter region of target genes revealed that mammalian (A) and _Drosophila_ (B) MTF-1 share the same core consensus and only seem to differ in some bases flanking the MREs. The consensus sequences were determined by alignment of 41 MREs originating from 16 mammalian target genes and 36 MREs originating from 9 target genes in _Drosophila_ using WebLogo [125]. Note that thymine residues preceding the MRE core consensus occur in a subset of _Drosophila_ MREs but are very rare in mammalian MREs. Nevertheless, such MREs were selected by human MTF-1 in an in vitro selection assay starting with random oligonucleotides under low zinc conditions [126], and might therefore confer particularly strong MTF-1 binding. Mammalian MREs are generally in a GC-rich background.

#### 3. MTF-1 target genes: too many responses for one factor?

The search for MTF-1 target genes other than metallothionein genes has been performed in human, mouse and _Drosophila_ systems (Table 1). Mammalian target genes with a role in metal homeostasis that are induced upon zinc load include _Slc30a1_ and _Slc30a2_ (solute carrier family 30 member 1 and 2, respectively). These encode the zinc exporters ZnT-1 and ZnT-2, respectively [61,62]. While MREs are typically located upstream, MTF-1 dependent expression of ZnT-2 is mediated by an MRE located 53 bp downstream of the transcription start [61]. Additionally MTF-1 represses transcription of _Slc39a10_ [63], which was subsequently shown to encode the zinc importer Zip10 [64]. In this case binding of MTF-1 to an MRE located 17 bp downstream of the transcription start results in transcriptional repression via RNA Pol II stalling [65].

Even though MTF-1 is not inducible by iron, a connection to this essential trace metal is given by the fact that expression of ferroportin 1, an iron export protein which shows also modest zinc export activity, is induced directly via MTF-1 [66]. Curiously, expression of hepcidin which is an inhibitor of ferroportin 1 was also shown to be induced by MTF-1 in a zinc-dependent manner via four MREs in its promoter [67]. Taken at face value, these data seem contradictory, but the induction thresholds or kinetics may differ.

A role for MTF-1 (and Sp1) was reported in copper-dependent upregulation of the gene encoding the prion protein PrP [68], which had long been implicated in copper homeostasis [69–72]. MTF-1 also activates expression of α-synuclein [73], another protein relevant to neurodegeneration and was postulated to counteract the aggregation of α-synuclein [74,75], which is typically found in Parkinson’s disease.

The role of MTF-1 in the defense against oxidative stress is underlined by the MTF-1 dependent expression of the selenoprotein 1 gene (Sepw1) which encodes a glutathione-binding protein with antioxidant activity [63]. However, MTF-1 is also involved in transcriptional
repression of the redox sensing selenoprotein H and the thioredoxin reductase 2 via MREs located downstream of the transcription start [76]. The gene for the catalytic subunit of glutamate–cysteine ligase (GCLC), an essential enzyme in glutathione biosynthesis, contains MREs in its promoter region and is downregulated in MTF-1 null mutant mouse embryos [33]. However the reduced transcript levels might primarily be a result of liver decay as glutathione levels remain high in embryos at earlier stage [77].

A link to the hypoxic stress response was established by the finding that MTF-1 contributes to the expression of placental growth factor (PIGF) [27], a member of the vascular endothelial growth factor family. MTF-1 activates the PIGF promoter in concert with the transcription factor NF-κB upon hypoxia [78]. MTF-1 also drives the cadmium-induced expression of N-myc downstream regulated gene 1 (NDRG1) [63], which encodes a protein of unknown molecular function. Interestingly however NDRG1 is induced by several stress conditions, is overexpressed in many types of cancer and its expression under hypoxic conditions is dependent on hypoxia-inducible factor 1 alpha (HIF-1α) [79]. A polymorphism in the promoter of the gene encoding dimethylarginine dimethylaminohydrolase 1 (DDAH1), that disrupts an MRE and leads to loss of MTF-1 binding, results in decreased DDAH1 mRNA levels and is associated with an increased risk of thrombosis stroke and coronary heart disease [80].

Target genes that might explain the lethal liver degeneration observed in MTF-1 null mutant mice are α-fetoprotein (AFP) and C/EBPα [77], both implicated in liver development in separate studies. Other genes that were shown to be regulated via MTF-1 without an obvious link to a stress response include the gene for cysteine- and glycine-rich protein 1 (Csrp1) [63], Krüppel-like factor 4 (KLF4), hepatitis A virus cellular receptor 1 (HAVCR1), complement factor B (CFB) [81] and tear lipocalin (LCN1) [77].

In insects, the search for MTF-1 target genes in the vinegar fly D. melanogaster yielded both similar and different targets: again the family of metallothionein genes emerged as major targets, as did Znt35C a zinc efflux transporter, CG10505 a homolog of the “yeast cadmium factor” and ferritin genes [35,82–84]. The major role of ferritins is in iron homeostasis, but they were also implicated in the sequestration of other metals including, Zn, Cu, Cd [85]. MTF-1 also increases the expression of the copper exporter DmaATP7 in larval midgut in response to copper load [86]. DmaATP7 is the Drosophila ortholog of the human copper transporters ATP7A and ATP7B. Mutations in these result in Menkes and Wilson’s disease, respectively, as a result of severe distortions of copper homeostasis [87]. Although MTF-1 typically induces target genes in response to metal load, in Drosophila it also induces transcription of the intestinal copper importer Ctr1B in response to copper starvation and is also needed for the repression of Ctr1B upon copper load [88]. Of note, in contrast to mammalian copper importers which are regulated post-transcriptionally, activity of Drosophila Ctr1B is mostly regulated at the transcriptional level [89].

Drosophila MTF-1, which also influences the expression of the copper exporter DmaATP7 [86], can be considered to be the main regulator
of copper homeostasis in the fly. The role of MTF-1 in copper homeo-
estasis in mammals is not well established. Copper availability does
not regulate the expression of the mammalian copper transporters
Ctri, ATP7A and ATP7B, rather their intracellular localization is changed
upon altered copper levels [90]. In line with mammalian MTF-1
having a minor role in copper homeostasis, zinc and cadmium induce a
very fast and robust transcription of MRE-containing reporter genes
in all cell types tested, while a response to copper is restricted to spec-
cific cell types (own observations). In mice a number of cadmium-
responsive genes were also found to be regulated independently of
MTF-1 and several of these are involved in glutathione metabolism.
MTF-1 null mutant cells are extremely sensitive to cadmium when
 glutathione synthesis is inhibited by treatment with buthionine sul-
fodoximide. This is strong evidence for two largely separate branches
of cadmium detoxification, namely, one via MTF-1 and the other via
 glutathione [63]. Post-transcriptional regulation of a group of zinc
transporters via a microRNA cluster was recently shown to represent
another MTF-1 independent mechanism in heavy metal homeostasis
[91].

It seems paradoxical that baker's yeast, for example, has three dif-
ferent heavy metal-responsive transcription factors (Mac1, Ace1,
Zap1) whereas higher organisms seem to rely only on MTF-1. Howev-
er, specificity of MTF-1 can be determined by the promoter architec-
ture of target genes and the interaction of different cofactors with
specific domains of MTF-1, as suggested by mutagenesis experiments
[58,59,88,92,93]. Furthermore, posttranscriptional regulation of metal
transporters also contributes to metal homeostasis.

4. Interaction partners of MTF-1: for better or worse

Considerable efforts have gone into the identification of MTF-1's in-
teraction partners (Table 2). Identified partners include basal
transcriptional coactivators such as TFIID and Mediator, as shown in
D. melanogaster [92]. This study also revealed an interesting aspect:
the promoters of Drosophila metallothioneins A and B, which show
preferential response to copper and cadmium, respectively [84], also
show differences in MTF-1 dependent coactivator recruitment [92].

An interactome study in Drosophila uncovered two novel interaction
partners [94]: Dpy-30L1 and Dpy-30L2. They are named this be-
cause of their sequence similarity to Dumpy-30 from C. elegans,
a small protein involved in chromatin modification. Dpy-30L1 inter-
feres with the binding of Drosophila MTF-1 to MREs. This interaction
is apparently insect-specific since it was not observed for human or
mammalian MTF-1 [95].

Mouse MTF-1 was found to form a zinc-induced complex with the
transcription factor Sp1 and the coactivator/histone acetyltransferase
p300. This interaction was mediated by the acidic activation domain
in mouse MTF-1 [93] and recruitment of p300 lead to chromatin
remodeling at the mouse metallothionein 1 promoter [96]. Interest-
ingly, chromium(VI) inhibits the formation of the p300/Sp1/MTF-1
complex on the mouse metallothionein 1 promoter and thereby im-
pairs RNA polymerase recruitment, suggesting a mechanism of chro-
mium toxicity [97]. We also found that human MTF-1 interacts with
p300 and serves as a substrate for acetylation via p300 in vitro. In con-
trast to mouse MTF-1, the interaction of human MTF-1 and p300 is
zinc independent (VG and WS, unpublished). The findings above
[93] suggest a positive role of Sp1 in metal induced transcription,
but this might not generally be the case since in other studies, Sp1
was reported to act as a negative regulator of the expression of
human metallothionein 2a [98,99].

The tumor suppressor protein PTEN was shown to bind to the
acidic activation domain of MTF-1 and to be important for MTF-1 de-
pendent metallothionein expression [100]. This interaction mainly
takes place in the cytoplasm and the protein phosphatase activity of
PTEN is necessary to activate MTF-1. This is consistent with previous
observations showing that MTF-1 activity is regulated by phosphory-
lation [101,102].

Besides interacting with transcriptional coactivators, several stud-
ies indicate that MTF-1 interacts with other stress responsive trans-
scription factors. The hypoxia-inducible factor 1 alpha (HIF-1α) was
shown to act in concert with MTF-1 to activate mouse metallothionein-1
transcription upon hypoxia [103]. Likewise, MTF-1 was shown to
promote HIF-1α dependent gene expression, since loss of MTF-1 attenuated HIF-1α nuclear accumulation and transcriptional
activity [29]. However, it remains to be seen whether this is due to
a direct effect of MTF-1 or to perturbation of hypoxia sensing by the
elevated glutathione levels in cells lacking MTF-1.

Several metallothionein genes contain AREs (antioxidant response
elements) next to MREs and are activated via the ARE-binding tran-
scription factors of the Nrf family [104].

Although metal-responsive promoters can be synergistically activ-
ated by heat and heavy metal stress, experimental alterations of
heat shock factor 1 (HSF1) concentrations had no effect on the tran-
scriptional activity of MTF-1 [105]. This argues against an involve-
ment of HSF1 in MTF-1/MRE dependent gene regulation. However,
the HSF1–MTF-1 interaction is relevant in the converse situation:
MTF-1 interacts with HSF1 and attenuates HSF1 mediated gene tran-
scription [106]. An additional connection between heat and metal
stress is given by the fact that cadmium induces the expression of cer-
tain heat shock proteins [107].

Another three transcription factor families have been reported to interact
with MTF-1 on metallothionein promoters: upstream stimulatory fac-
tor 1 (USF1), nuclear factor 1 (NF1) and CCAAT/enhancer-binding
protein alpha (C/EBPα). USF1 upregulates transcription of male-

| **Table 2**

Main interaction partners of mammalian and *Drosophila* MTF-1.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Organism</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>TFIID complex</td>
<td><em>Drosophila</em></td>
<td>Subunits mostly inhibit initiation of transcription of the metallothioneinA (MtnA) gene [92]</td>
</tr>
<tr>
<td>Mediator complex</td>
<td><em>Drosophila</em></td>
<td>Different subunits are recruited to the promoters of the MtnA, MtnB and MtnD genes; stimulation of transcription [92]</td>
</tr>
<tr>
<td>Dpy30L1 (and Dpy30L2)</td>
<td><em>Drosophila</em></td>
<td>Direct interaction; negative effect by inhibition of DNA binding, potential chromatin modulator [94]</td>
</tr>
<tr>
<td>Sp1</td>
<td>Mouse/human</td>
<td>Direct interaction; complex results: activates transcription of mouse MT-1, but inhibits transcription of human MT-2α [93]</td>
</tr>
<tr>
<td>p300/CBP</td>
<td>Mouse/human</td>
<td>Direct interaction; contributes to induction of mouse MT-1, but not Ztn1; MTF-1 is acetylated by p300 in vitro [98]</td>
</tr>
<tr>
<td>PTEN</td>
<td>Human/mouse</td>
<td>Direct interaction in the cytoplasm; activates human MT-2α, MT-1α and Ztn1 expression [100]</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>Mouse</td>
<td>Direct interaction; cooperates with MTF-1 to induce MT-1 in response to hypoxia [103]</td>
</tr>
<tr>
<td>Nrf1</td>
<td>Mouse</td>
<td>Nrf1 and MTF-1 cooperate to activate basal MT-1 and MT-2 expression in mouse liver [104]</td>
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<tr>
<td>HSF1</td>
<td>Human</td>
<td>MTF-1 reduces metal-induced HSF1-dependent Hsp70 expression [105]</td>
</tr>
<tr>
<td>USF1</td>
<td>Mouse</td>
<td>MTF-1 and USF1 cooperate to activate basal MT-1 expression in the visceral yolk sac [108]</td>
</tr>
<tr>
<td>NF1</td>
<td>Mouse</td>
<td>Contradicting results: NF1 is reported to either enhance or inhibit MTF-1 activity at the MT-1 promoter [109]</td>
</tr>
<tr>
<td>C/EBPα</td>
<td>Human</td>
<td>Direct interaction; C/EBPα stimulates MT-2α expression [111]</td>
</tr>
</tbody>
</table>

[*] Own observations.
metallothionein 1 in concert with MTF-1 [108]. The role of NF1 remains to be clarified. In one study it was shown to repress metallothionein 1 expression [109] whereas another one suggested that NF1 requires MTF-1 for zinc dependent binding to the promoter to activate transcription [110]. MTF-1 also cooperates with the product of one of its target genes: C/EBPβ, a tumor suppressor protein enriched in the liver, positively regulates metallothionein 2α expression in human hepatoma (HEP3B) cells [111].

5. Concluding remarks/outlook

The role of MTF-1 in metal homeostasis is well established—it can upregulate expression of exporters and scavengers and repress expression of importers in response to heavy metal exposure. Evidence also points to a role in other stress response pathways, such as hypoxia and oxidative stress caused by reactive oxygen species, and in neuroprotection. The interaction partners of MTF-1 also support the notion that MTF-1 is interconnected with multiple cell stress response pathways. At the risk of oversimplification one could conclude that each cell stress response is typically coordinated by a major, stress specific transcription factor but also involves to a lesser extent regulators of other types of stress.

With its central role in protecting a cell from diverse stressors it is not surprising that MTF-1 is implicated in a diverse set of human diseases. Regarding cancer, MTF-1 can be expected to have a beneficial role for the organism by counteracting pro-cancerogenic conditions, notably heavy metal and oxidative stress. However once a tumor emerges, MTF-1 can promote cancer cell survival by enhancing expression of metallothioneins, stimulation of angiogenesis and extracellular matrix remodeling [112,113]. In line with this fact MTF-1 was shown to be upregulated in some tumor cells [114]. Resistance of lymphoma cells to gallium nitrate is partially mediated by MTF-1/metallothionein [115]. Furthermore, a polymorphism in the mouse MTF-1 gene was implicated in the development of γ-ray induced lymphomas [116]. Of particular interest are the findings in Drosophila models of neurodegenerative diseases, namely Alzheimer’s and Parkinson’s disease [117,118], which are both characterized by increased oxidative stress and heavy metal sensitivity in affected neurons [119].

There, elevated expression of MTF-1 dramatically improves the condition of parkin null mutant flies or transgenic flies expressing human amyloid beta peptide (Aβ/42) [117,118]. Aβ/42 is the main component of amyloid plaques found in brains of Alzheimer’s patients.

Although many new findings have widened our view on MTF-1’s role in heavy metal homeostasis and detoxification and in other cell stress responses, major questions remain. How does MTF-1 activate transcription of most of its target genes but contributes to the repression of others, such as mouse selenoprotein H and Drosophila Ctr1B? In the glucocorticoid hormone response, activation or repression are known to be dependent on the DNA sequence of the glucocorticoid receptor binding sites [120]. With MTF-1, both positive and negative responses seem to be mediated via the same MRE consensus sequence; the output rather depends on their spatial arrangement [88] which is thought to dictate interactions with coactivators and co-repressors. In the case of the Drosophila copper importer Ctr1B, the strong MRE sites located around position -700 from the transcription start might allow MTF-1 to bind even under copper-replete conditions, thus allowing transcription at low copper. Upon heavy metal load the Ctr1B gene is repressed, possibly via interaction of MTF-1 with a co-repressor [88]. The inhibitory C-terminal domain plays an important role because following its deletion MTF-1 indiscriminately activates both metallothionein and Ctr1B expression in normal food. This is remarkable because other mutations of Drosophila MTF-1 typically hamper metallothionein induction by metal without affecting Ctr1B expression.

Elusive is also the exact mechanism of metal sensing by MTF-1 and whether it is always direct or mediated in concert with other proteins. For mammalian MTF-1 the zinc fingers and the acidic activation domain were shown to mediate zinc sensing, whereas the acidic activation domain works as an independent metal-inducible unit at least in a subset of cell types [52]. The response to other heavy metals, to oxidative stress and to nitric oxide might be an indirect one, mediated mainly by release of zinc from metallothioneins and possibly other metal binding proteins [121–123]. However this model is certainly not covering all aspects of metal induction, and additional mechanisms are contributing (this review, and see also [124]).

Metal specificity is another enigma: how does the cell, always in an MTF-1 dependent manner, ensure the differential response of certain genes to one but not the other metal? One clue might come from the finding that for two metallothionein promoters which are preferentially induced by copper or cadmium (Drosophila MtnA and MtnB, respectively), activation by MTF-1 involves differential recruitment of cofactors and Mediator components [92].

Also unclear is the role of the nuclear export signal, since at least with human MTF-1, export per se is not essential to regulate metal inducibility. It might be necessary for full efficiency and/or another function of MTF-1. Thus, it would be interesting to see whether genes repressed by MTF-1, like Zip10, are permanently repressed by an MTF-1 with constitutive nuclear localization, for example as a result of a mutated, dysfunctional nuclear export signal.

We are confident that in the years to come, these open questions will be successfully addressed by the combined efforts of multiple laboratories.

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References


