The DNA Binding Activity of Metal Response Element-binding Transcription Factor-1 Is Activated in Vivo and in Vitro by Zinc, but Not by Other Transition Metals*

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We examined the DNA binding activity of mouse and human MTF-1 in whole cell extracts from cells cultured in medium containing zinc or cadmium and from untreated cells after the in vitro addition of zinc or cadmium, as well as using recombinant MTF-1 transcribed and translated in vitro and treated with various transition metals. Incubation of human (HeLa) or mouse (Hepa) cells in medium containing cadmium (5–15 μM) did not lead to a significant increase (<2-fold) in the amount of MTF-1 DNA binding activity, whereas zinc (100 μM) led to a 6–15-fold increase within 1 h. MTF-1 binding activity was low, but detectable, in control whole cell extracts and was increased (>10-fold) after the in vitro addition of zinc (30 μM) and incubation at 37 °C for 15 min. In contrast, addition of cadmium (6 or 60 μM) did not activate MTF-1 binding activity. Recombinant mouse and human MTF-1 were also dependent on exogenous zinc for DNA binding activity. Cadmium did not facilitate activation of recombinant MTF-1, but instead inhibited the activation of the recombinant protein by zinc. Interestingly, glutathione (1 mM) protected recombinant MTF-1 from inactivation by cadmium, and allowed for activation by zinc. It was also noted that zinc-activated recombinant MTF-1 was protected from cadmium only when bound to DNA. These results suggest that cadmium interacts with the zinc fingers of MTF-1 and forms an inactive complex. Of the several transition metals (zinc, cadmium, nickel, silver, copper, and cobalt) examined, only zinc facilitated activation of the DNA binding activity of recombinant MTF-1. These data suggest that transition metals, other than zinc, that activate MT gene expression may do so by mechanisms independent of an increase in the DNA binding activity of MTF-1.

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Metallothioneins (MT)1 constitute a conserved family of cysteine-rich heavy metal-binding proteins (1). In the mouse, MT-I and MT-II display a wide tissue distribution and have been demonstrated to participate in detoxification of transition metals such as cadmium (2, 3), zinc homeostasis (4), and protection against oxidative stress (5). MT-I and MT-II gene transcription is induced dramatically by heavy metals (especially zinc and cadmium) (6). Metal response elements (MRE) are essential for this induction, and these elements are present in multiple copies in the proximal promoters of MT genes. MREs were initially shown to mediate transcriptional response of MT genes to zinc and cadmium (7–9), and more recently to oxidative stress (10, 11).

A protein that binds specifically to MREs and that transactivates MT gene expression has been cloned from mouse and human, and is termed MTF-1 (MRE-binding transcription factor-1) (12, 13). MTF-1 is a zinc finger transcription factor in the Cys2-His2 family. The DNA binding activity of MTF-1 is reversibly regulated by zinc interactions with the zinc finger domain (14). In contrast with some zinc finger proteins, including zinc transcription factors, which can bind zinc with picomolar to nanomolar dissociation constants (15, 16), MTF-1 is regulated by micromolar concentrations of this metal. Thus, MTF-1 may serve as a sensor for “free” zinc within the cell. Manipulation of MTF-1 expression by targeted deletion of both genes in embryonic stem cells (17) or by expression of antisense MTF-1 (18) eliminates metal responsiveness of transfected MRE-driven reporter genes. Thus, MTF-1 is thought to be essential for activation of MRE activity by all of the transition metals that have been examined.

Although MTF-1 may play a role in activating MT gene expression in response to several transition metals, the nature of the interaction between MTF-1 and metals other than zinc has not been examined. To clarify the mechanisms of activation of MTF-1 by transition metals, we examined the DNA binding activity of MTF-1 in vivo in cells treated with cadmium, and we utilized whole cell extracts prepared from mouse and human cells and recombinant mouse and human MTF-1, transcribed and translated in vitro, to study effects of transition metals on MTF-1 DNA binding activity. We found that MTF-1 DNA binding activity is poorly activated in vivo by cadmium, and that it is activated in vitro by zinc but not by any of the other transition metals tested. These data suggest that transition metals, other than zinc, activate MT gene expression through mechanisms independent of a significant increase in DNA binding activity of MTF-1.

1 The abbreviations used are: MT, metallothionein; MRE, metal response element; DTT, dithiothreitol; EMSA, electrophoretic mobility shift assay; PAGE, polyacrylamide gel electrophoresis.
EXPERIMENTAL PROCEDURES

Cell Culture—Mouse Hepa cells were maintained in Dulbecco's modified Eagle's medium-high glucose supplemented with 2% fetal bovine serum. HeLa cells were maintained in RPMI 1640 medium with 10% fetal bovine serum, 100 units/ml penicillin, and 50 μg/ml streptomycin, and canine Madin-Darby kidney cells (American Type Culture Collection, Rockville, MD) were cultured in Dulbecco's modified Eagle's medium-high glucose supplemented with 10% fetal bovine serum.

Preparation of Whole Cell Extracts—Whole cell extracts were prepared as described previously (19), with modifications (14). Cells were lysed by suspension in 3 volumes of extraction buffer (20 mM Hepes (pH 7.9), 1.5 mM MgCl₂, 400 mM KCl, 0.5 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride, and 25% glycerol) and centrifuged at 89,000 × g for 5 min. The supernatant was collected and stored in aliquots at −80 °C. Protein concentrations were determined using a Bradford protein assay (Bio-Rad) using rabbit IgG as a standard.

In Vitro Transcription/Translation of Mouse and Human Recombinant MTF-1—The mouse MTF-1 cDNA clone was described previously (14), whereas that for human MTF-1 was a generous gift from Dr. Walter Schaffner, University of Zurich, Switzerland. Human MTF-1 cDNA was amplified by polymerase chain reaction using flanking primers and subcloned into the XhoI site of the pcDNA3 plasmid (Invitrogen Corp., Palo Alto, CA). Recombinant MTF-1 was synthesized in vitro using a TnT Coupled Reticulocyte Lysate Transcription/Translation System (Promega Biotech), containing 1 μg of the MTF-1 plasmid and Sp6 (mouse MTF-1) or T7 (human MTF-1) RNA polymerase according to the manufacturer's suggestions.

Electrophoretic Mobility Shift Assay—EMSA was performed as described (11, 14). Proteins from whole cell extracts (20 μg) or the MTF-1 in vitro transcription/translation reaction (1 μl of a 50-μl reaction) were incubated in buffer containing 12 mM Hepes (pH 7.9), 60 mM KCl, 0.5 mM DTT, 12% glycerol, 5 mM MgCl₂, 0.2 μg of poly(dI-dC) of protein, 2–4 fmol of end-labeled MRE-s double-stranded oligonucleotide (5000 cpm/fmol) in a total volume of 20 μl (20) as described in the figure legends and under "Results." Effects of the addition of exogenous metals on MTF-1-MRE-s complex formation were examined, as were effects of incubation temperature, time, and the concentration of reducing agent. ZnSO₄, CdCl₂, CoCl₂, CuSO₄, NiCl₂, AgNO₃, were dissolved in acidified H₂O as 1000 times concentrated stock solutions. Glutathione (GSH) was dissolved fresh as a 20 mM solution in 50 mM Tris-HCl (pH 8.0). The oligonucleotide sequences used were as follows; bold bases denote the functional core:

5′-GGCCCGAAAAG-TAGTCCCTCAGAGGACTTGCAACCGGCCGCAAAGTAGTCCCTCGAGACGTGTGCCGGGCTTTTCATCTAG-3′

Gels were dried and labeled MRE-s or Sp1 were detected by autoradiography. The specific complexes are indicated by arrows. The Sp1 oligonucleotide has two binding sites which give rise to two predominant bands during EMSA.

RESULTS

The DNA Binding Activity of Native MTF-1 Is Activated by Zinc, but Not by Cadmium Both in Vivo and in Vitro—MTF-1 binding activity was examined in whole cell and nuclear extracts by EMSA using a consensus MRE oligonucleotide (MRE-s) that has a specific, high affinity MTF-1-binding site (12). Several lines of evidence have established that MTF-1 is a component of the complex formed with MRE-s in whole cell and nuclear extracts (11, 14). This complex is absent from embryonic stem cells lacking MTF-1 (22) and is restored after transfection with a mouse MTF-1 expression plasmid (14). The mobility shift, sequence specificity, and zinc dependence of this complex are indistinguishable from that of recombinant MTF-1.

Our previous studies documented that the DNA binding activity of MTF-1 is positively regulated both in vivo and in vitro by zinc. In contrast, the effects of other transition metals on the DNA binding activity of MTF-1 have not been examined. A modest activation (~2-fold) of MTF-1 binding activity was detected in whole cell extracts from Hepa cells incubated in medium containing 6 or 60 μM cadmium (14). Cadmium is a 5–10-fold more potent inducer of MT mRNA than zinc (23, 24), and 6 μM cadmium dramatically induced MT-I mRNA in these cells (data not shown), as does 60 μM zinc (10). Whole cell extracts from mouse Hepa and human HeLa cells that had been incubated for 1 or 4 h, as indicated, in medium containing 60 μM zinc or 6 μM cadmium. Aliquots of whole cell extracts (20 μg of protein) were analyzed by EMSA using a labeled MRE-s oligonucleotide (part A) or a labeled Sp1 oligonucleotide (part B). Binding reactions were incubated on ice for 20 min and subjected to PAGE. Gels were dried and labeled MRE-s or Sp1 were detected by autoradiography. The specific complexes are indicated by arrows. The Sp1 oligonucleotide has two binding sites which give rise to two predominant bands during EMSA.

MTF-1 binding activity can be dramatically increased in vivo by zinc, and 6 μM cadmium dramatically increased MTF-1 binding activity in nuclear extracts from Hepa cells incubated in medium containing 6 or 60 μM cadmium (14). Cadmium is a 5–10-fold more potent inducer of MT mRNA than zinc (23, 24), and 6 μM cadmium dramatically increased MT-I mRNA in these cells (data not shown), as does 60 μM zinc (10).

FIG. 1. In vivo activation of MTF-1 DNA binding activity in mouse Hepa and human HeLa cells by zinc, but not by cadmium. Whole cell extracts were prepared from mouse Hepa cells and human HeLa cells that had been incubated for 1 or 4 h, as indicated, in medium containing 60 μM zinc or 6 μM cadmium. Aliquots of whole cell extracts (20 μg of protein) were analyzed by EMSA using a labeled MRE-s oligonucleotide (part A) or a labeled Sp1 oligonucleotide (part B). Binding reactions were incubated on ice for 20 min and subjected to PAGE. Gels were dried and labeled MRE-s or Sp1 were detected by autoradiography. The specific complexes are indicated by arrows. The Sp1 oligonucleotide has two binding sites which give rise to two predominant bands during EMSA.
Zinc-specific Activation of MTF-1

In vitro by the addition of low micromolar concentrations of zinc to whole cell extracts from control (untreated) cells (14). This activation process is time and temperature dependent and is maximal in whole cell extracts containing exogenous zinc (30 μM) after incubation at 37 °C for 15 min. The effects of cadmium on the binding activity of MTF-1 were examined using whole cell extracts from untreated Hepa, HeLa, and canine Madin-Darby kidney cells (Fig. 2A). The indicated concentrations of zinc and/or cadmium were added to the EMSA binding reactions, and the reactions were incubated at 37 °C for 15 min before addition of labeled MRE-s and subsequent electrophoresis. Under these conditions, cadmium, even at high (60 μM) concentration, had no effect on MTF-1 binding activity. In contrast, as little as 2 μM zinc caused a detectable activation of MTF-1 and 30 μM zinc resulted in a >10-fold activation. Furthermore, cadmium did not appreciably affect the ability of zinc to activate MTF-1 in vitro in these whole cell extracts discounting the possibility that cadmium irreversibly denatures MTF-1 (Fig. 2A). In vitro activation of MTF-1 by zinc is temperature sensitive, so we examined the effects of temperature on cadmium (6 μM) activation of MTF-1 binding activity (Fig. 2B). Again, cadmium failed to cause activation of MTF-1 in Hepa whole cell extracts, regardless of the incubation temperature examined (4–37 °C). Remarkably, cadmium did not reduce the constitutive Sp1 binding activity in these whole cell extracts (Fig. 2C).

The DNA Binding Activity of Recombinant MTF-1 Is Activated by Zinc, but Not by Cadmium, and Higher Concentrations of Cadmium Interfere with Zinc Activation—The effects of zinc and cadmium on mouse and human MTF-1 binding activity were examined using recombinant MTF-1 produced in a coupled transcription/translation system (TnT lysate). As reported previously (14), mouse MTF-1 synthesized in the TnT reaction exhibits little or no DNA binding activity before the addition of exogenous zinc (30 μM) followed by incubation at 37 °C for 15 min. This was also the case for human MTF-1, and half-maximal activation of recombinant mouse and human MTF-1 required 2–3 μM zinc under these conditions (Fig. 3A). In contrast, cadmium (3 μM) did not lead to activation of MTF-1, nor did it inhibit the ability of zinc to activate this DNA binding activity (Fig. 3B, data for mouse MTF-1 are shown). However, it was noted that activation of recombinant MTF-1 by zinc (30 μM) was reduced by 60% when 6 μM cadmium was included during the activation process (37 °C for 15 min) (Fig. 3C, lane 3). Higher concentrations of cadmium (30 μM) completely prevented zinc activation of MTF-1 (Fig. 3D, lanes 4 and 5). Similar results were obtained using recombinant human MTF-1 (data not shown). The TnT lysate contains 18.8 ± 3.7 μM total zinc (14). Therefore, these EMSA reactions contained about 2 μM zinc. If cadmium had caused the redistribution of this zinc, then the MTF-1 binding activity would have been half-maximal.

Effects of cadmium on recombinant MTF-1 that had been previously activated with zinc were examined. Zinc-activated mouse MTF-1 or mouse MTF-1-MRE-s complexes were incubated with cadmium. Relative to free mouse MTF-1, pre-existing mouse MTF-1-MRE-s complexes were significantly more resistant to cadmium. Pre-existing mouse MTF-1-MRE-s complexes were resistant to 6 μM cadmium (Fig. 3C, compare lanes 2 and 3) and 30 μM cadmium (Fig. 3D, compare lanes 4 and 5). In contrast, 60 μM cadmium dramatically reduced the amount of complex detected regardless of whether the protein was

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**Fig. 2. In vitro activation of MTF-1 DNA binding activity by zinc, but not by cadmium, in whole cell extracts from untreated mouse, human, and canine cells.** Whole cell extracts (20 μg/reaction) from untreated mouse Hepa, human HeLa, or canine Madin-Darby kidney cells were adjusted to the indicated final concentrations of exogenous zinc, cadmium, or both. Binding reactions were incubated at 37 °C for 15 min, labeled MRE-s was added and the incubation was continued at 4 °C for 20 min. All binding reactions were then subjected to PAGE. Gels were dried and labeled MRE-s was detected by autoradiography. Shown here is only that region of each gel containing the MTF-1-MRE-s complex (part A) or the entire gel with this complex indicated by an arrow (part B). The Sp1 complexes are indicated by arrows (part C).
Zinc-specific Activation of MTF-1

The binding reaction containing 30 \( \mu M \) zinc served as the 100% activation standard and values shown represent the average \( \pm \) S.E. of three determinations. Part B, binding reactions contained labeled MRE-s oligonucleotide and, as indicated, zinc (30 \( \mu M \)) and/or cadmium (3 \( \mu M \)). Reactions were incubated at 37 °C for 15 min, and then at 4 °C for 20 min before PAGE. In the absence of exogenous zinc or if MTF-1 plasmid was omitted, no MTF-1 binding activity was detected by EMSA. Part A, the effects of zinc concentration on the DNA binding activity of mouse MTF-1 (mMTF) or human MTF-1 (hMTF) were examined. Binding reactions (14 \( \mu l \)) were assembled with 0.5 \( \mu l \) of the TnT lysates and the indicated concentrations of exogenous zinc. Binding reactions were incubated at 37 °C for 15 min, placed on ice, and labeled MRE-s oligonucleotide was added. After 15 min at 4 °C, the reactions were subjected to PAGE. The amount of MTF-1-MRE-s complex was quantitated by PhosphorImager analysis. Reactions containing 30 \( \mu M \) zinc served as the 100% activation standards and values shown represent the average \( \pm \) S.E. of three determinations. Part B, binding reactions contained labeled MRE-s oligonucleotide and, as indicated, zinc (30 \( \mu M \)) and/or cadmium (3 \( \mu M \)). Reactions were incubated at 37 °C for 15 min, and then at 4 °C for 20 min before PAGE. In the absence of exogenous zinc or if MTF-1 plasmid was omitted, no MTF-1 binding activity was detected (14). Part C, binding reactions containing recombinant mouse MTF-1 were adjusted to 30 \( \mu M \) zinc and incubated at 37 °C for 15 min (lanes 1–3). In lane 2, the zinc-activated MTF-1 was then allowed to bind to MRE-s, and 6 \( \mu M \) cadmium was subsequently added and the mixture was incubated at 37 °C for 15 min. In lane 3, 6 \( \mu M \) cadmium was added at the same time as zinc. In lane 4, MTF-1 was incubated with 6 \( \mu M \) cadmium alone. Labeled MRE-s was then added and these reactions (lanes 2–4) were incubated at 4 °C for 20 min before PAGE. The arrow indicates the specific MTF-1-MRE-s complex. The amount of complex was quantitated by PhosphorImager analysis. Part D, the effect of cadmium on pre-existing zinc-activated MTF-1 and MTF-1-MRE-s complexes was investigated. The binding reaction containing 30 \( \mu M \) zinc served as a comparative standard (lane 1). MTF-1 was activated with 30 \( \mu M \) zinc and allowed to bind to MRE-s. 60 \( \mu M \) cadmium (lane 2) or 30 \( \mu M \) cadmium (lane 4) was subsequently added and the mixture incubated at 37 °C for 15 min. In parallel samples, MTF-1 was activated with 30 \( \mu M \) zinc, as above, and then 60 (lane 3) or 30 (lane 5) \( \mu M \) cadmium was added and the mixture incubated at 37 °C for 15 min. Labeled MRE-s was added and samples were analyzed by PAGE. The arrow indicates the specific MTF-1-MRE-s complex.
Zinc-specific Activation of MTF-1

The zinc finger transcription factor MTF-1 has been suggested to play an essential role in induction of MT gene expression by several transition metals (17, 18). Among these transition metals, zinc and cadmium are the most effective inducers of MT gene expression in mouse cells. The dose-response for induction of MT genes and MRE-driven reporter genes by cadmium is 5–10-fold lower than that for zinc. We recently reported that zinc can reversibly and directly activate the DNA binding activity of mouse MTF-1 (14), and the amount of MTF-1 DNA binding activity, measured in vitro, is dramatically and rapidly increased after treatment of cells with zinc or oxidative stress-inducing agents (11). This increased MTF-1 activity correlates with increased occupancy of MREs in the MT-I promoter (11). Interestingly, the efficacy of induction by many transition metals, of MRE-driven reporter gene expression in transfected cells, is diminished when the concentration of zinc in the culture medium is reduced. Therefore, it has been suggested that zinc mediates induction of MT gene expression by other transition metals (18). Furthermore, genomic footprinting suggests that cadmium increases the occupancy of MREs in MT promoters in cultured cells (28) which implies that cadmium activates the DNA binding activity of MTF-1 in vivo. Therefore, we examined MTF-1 DNA binding activity in cells treated with zinc or cadmium, and of recombinant MTF-1 synthesized in a coupled transcription-translation system and exposed to various transition metals in vitro.

In contrast with oxidative stress and zinc (11, 14), cadmium has little effect on the amount of MTF-1 DNA binding activity extracted from Hepa cells cultured in medium containing cadmium. Furthermore, the modest increase in MTF-1 activity that we reported previously (14) was detected later than induction of MT-I gene expression by cadmium in these cells. The results reported herein confirm and extend our previous conclusion that cadmium, in concentrations that rapidly and efficiently induce MT gene expression, does not cause the rapid activation of MTF-1 DNA binding activity in mouse, human, and dog cells. These results suggest that cadmium does not simply cause the redistribution of zinc which, in turn, rapidly

**DISCUSSION**

binant MTF-1—Many transition metals have been reported to induce MT gene expression (17, 18), and it has been suggested that some of these metals may cause the redistribution of zinc, thus leading to activation of MTF-1 (18). To determine whether any of these metals can directly activate MTF-1, the effects of nickel, silver, copper, and cobalt on the DNA binding activity of recombinant MTF-1 were determined. Each metal ion was tested at several concentrations, ranging from 3 to 500 μM, in the presence or absence of 1 mM GSH. Each metal at higher concentrations inhibited the zinc activation of MTF-1. However, the effective inhibitory concentration was metal-specific, and in each case 1 mM GSH provided significant protection (see Fig. 5). Of the metals examined, cadmium, copper and nickel were inhibitory in the lowest concentrations to zinc activation of MTF-1. Using the maximal concentration of each metal ion

**FIG. 5.** In vitro activation of the DNA binding activity of recombinant mouse MTF-1 by zinc, but not by other transition metals. Recombinant mouse MTF-1 was synthesized in vitro in a TnT lysate programmed with mouse MTF-1 (14) plasmid. The DNA binding activity of MTF-1 was detected by EMSA using labeled MRE-s. Binding reactions all contained GSH (1 mM), and zinc (30 μM), and/or the indicated concentration of each transition metal. Reactions were incubated at 37 °C for 15 min and then labeled MRE-s was added and the incubation was continued at 4 °C for 20 min before PAGE. The arrow indicates the specific MTF-1-MRE-s complex.

**Fig. 4.** GSH can protect mouse and human recombinant MTF-1 from cadmium inactivation of DNA binding activity. Recombinant mouse and human MTF-1 were synthesized in vitro in a TnT lysate programmed with mouse MTF-1 (14) or human MTF-1 (13) plasmid. The DNA binding activity of MTF-1 was detected by EMSA. Part A, recombinant mouse MTF-1 was examined. Binding reactions contained labeled MRE-s oligonucleotide and 30 μM zinc, plus GSH (1 or 10 mM) and/or cadmium (6 or 30 μM), as indicated. Reactions were incubated at 37 °C for 15 min, and then labeled MRE-s was added and the incubation continued at 4 °C for 20 min before PAGE. The arrow indicates the specific MTF-1-MRE-s complex. Part B, recombinant mouse MTF-1 (mMTF-1) and human MTF-1 (hMTF-1) were analyzed by EMSA using labeled MRE-s. Binding reactions were adjusted to contain zinc (30 μM), zinc plus GSH (1 mM), cadmium (6 μM) plus GSH, or zinc and cadmium plus GSH, as indicated. Reactions were incubated at 37 °C for 15 min and then at 4 °C for 20 min before PAGE. The arrow indicates the specific MTF-1-MRE-s complex.
activates MTF-1 to bind to DNA. Our results cannot exclude
the possibility that cadmium causes an increase in the binding
affinity of a small amount of MTF-1. This might explain the
increased occupancy of MREs detected in vivo after treatment
of cultured cells with cadmium (28). Due to the difficulty in
detecting the small amount of MTF-1 binding activity in the
cadmium-treated cells, it was not possible to accurately com-
pare the affinity of binding of this transcription factor in cad-
mium-treated versus zinc-treated cells.

Recombinant human and mouse MTF-1 synthesized in vitro
in a coupled transcription-translation system is not competent
to bind to DNA, but can be activated by low micromolar con-
centrations of zinc (2–3 μM) in a temperature-dependent man-
er. Examination of the ability of several transition metals,
including cadmium, to activate recombinant MTF-1 in vitro
revealed that none of these metal ions, other than zinc, directly
activated this transcription factor to bind to DNA. The TnT
lysate contributed 2 μM zinc to the EMSA reactions, which if
freed would be expected to cause half-maximal activation of
MTF-1. However, similar to the whole cell extracts which con-
tained significant levels of zinc, cadmium and other transition
metals did not activate MTF-1. A wide range of metal concen-
trations was examined in these experiments. These results are
consistent with the hypothesis that transition metals that ac-
tivate MT gene expression do so by indirect mechanisms inde-
pendent from those used by zinc.

Unlike recombinant human and mouse MTF-1 which display
no binding activity in the absence of exogenous zinc, in cultured
cells about 10% of the MTF-1 extracted is active to bind to DNA
in the absence of exogenous zinc (11). This constitutive activity
does not result from activation during extraction since at 4 °C
even high concentrations of exogenous zinc do not activate the
DNA binding activity of MTF-1 (14). The presence of a basal
amount of activated MTF-1 in the cell is perhaps not surprising
since it has been hypothesized that maintenance of zinc home-
ostasis within the cell involves not only the efflux of zinc by
specific transporters (29, 30), but also a balance between zinc-
dependent MTF-1 activation of MT gene transcription and the
synthesis of the major intracellular zinc-binding protein MT.
This scenario is analogous to the control of copper homeostasis
in yeast by the copper-dependent transcription factor ACE1 and
the yeast MT, CUP1 (31, 32). Since all transition metals
that activate mammalian MT gene expression are thought to
do so via MTF-1, and with the exception of zinc, without a
significant increase in MTF-1 binding activity, our data sug-
gest that these transition metals make use of the activated
MTF-1 that pre-exists in the cell to increase MT gene expres-
sion. This notion is indirectly supported by the observation that
zinc concentration in the culture medium influences the extent
of induction of an MRE-driven reporter gene by other transition
metals, including cadmium (18). The mechanism by which
transition metals other than zinc act on MTF-1 to increase MT
gene expression is poorly understood.

It is possible that transition metals alter the transactivation
potential of MTF-1 by releasing an inhibitor or activating a
co-activator. We speculate that reversible phosphorylation of
MTF-1 may play a role in modulating the activities of this
transcription factor. Cadmium can stimulate myosin light
chain kinase (33), affect calmodulin activity in the brain (34),
and evoke inositol polyphosphate formation (35). Cadmium
induction of e-nuc may involve the activation of protein kinase
C (36). Cadmium may modulate gene expression by interfering
with normal cellular signaling mechanisms at the levels of
receptors, calcium and zinc homeostasis, protein phosphoryla-
tion, and modification of transcription factors (37). Mouse
MTF-1 contains a serine/threonine-rich transactivation do-
main (22), and several potential sites of phosphorylation, but
no studies of the phosphorylation state of the protein have been
reported.

The proximal promoter of the mouse MT-I gene contains a
complex array of transcriptional activation elements, and al-
though it has been shown that MTF-1 is essential for basal
level expression of MT, as well as heavy metal induction (17,
18), it seems likely that MTF-1 may function in cooperation
with other transcription factors to regulate MT gene expres-
sion. Therefore, induction of MT by heavy metals other than
zinc may involve the enhanced interaction of other transcrip-
tion factors with MTF-1 in the absence of an increase in MTF-1
DNA binding activity. It is clear that MTF-1 plays an integral
role in transducing a complex cascade of signals resulting in
the activation of MT gene transcription. Determining the na-
ture of the mechanism by which heavy metals initiate this
cascade requires further investigation.

Rather than activate the DNA-binding capacity of recombi-
nant MTF-1, it was noted that, in vitro, several transition
metals actually inhibited the activation of recombinant MTF-1
by zinc. Cadmium was much less efficient at inhibiting pre-
formed MTF-1-DNA complexes than it was at inhibiting free
MTF-1. We (11) and others (12) have noted that zinc-activated
MTF-1 bound to MRE-s contains a proteinase-resistant DNA-
binding domain. Furthermore, relative to Sp1, MTF-1 binding
activity is more sensitive to EDTA, and is stabilized by DNA
interactions (14). These data, and deletion mutagenesis exper-
iments, suggest that the reversible interactions of zinc with the
zinc finger domain of MTF-1 modulates its DNA binding activ-
ity. Other transition metals likely inhibit zinc binding to
MTF-1 zinc fingers (16). Consistent with this notion, it was
found that GSH, in physiological concentrations (1 mM), and
low levels of DTT or β-mercaptoethanol, provided protection
against the detrimental effects of transition metals on zinc
activation of MTF-1, whereas, higher concentrations (10 mM)
of reducing agents prevented the activation of recombinant
MTF-1 by zinc. Thus, the redox status of thiols in MTF-1 may
be critical for zinc activation of DNA binding activity. Alterna-
atively, or in addition, the effects of GSH may reflect its metal
binding ability (25, 38). GSH binds cadmium with an equilib-
rium constant of 2 × 10^{-10} M and zinc with an equilibrium
constant of 2 × 10^{-8} M (38). At higher concentrations (10 mM)
GSH may simply chelate zinc and prevent activation of MTF-1.
In lower concentrations (1 mM), GSH may preferentially bind
cadmium and prevent its interactions with MTF-1. In contrast,
the protective effects of DTT and β-mercaptoethanol are un-
likely to reflect their metal binding capacity. In vivo, MTF-1
can be protected from inactivation by transition metals by the
reducing environment within the cell and/or by the binding of
these metals by metallothionein (39).

Several transition metals (cadmium, cobalt, copper, iron,
manganese, mercury, nickel, and zinc) can bind to zinc finger
motifs (reviewed in Ref. 16). These motifs fold in the presence
of transition metal ions but not in their absence. Metal binding
constants are governed by the geometry of the binding site and
by the amino acid sequence of the zinc finger (15, 40). Zinc is
bound with the highest affinity of the transition metals (40).
The DNA binding activity of the Cys_2Cys_2 zinc finger domains
from the glucocorticoid receptor (41) and GAL4 (42) can be
restored by cadmium and/or cobalt. In contrast, the DNA
binding activity of Cys_2His_2 zinc finger protein TFIIA is not re-
stored by cobalt, nickel, or iron, but can be partially restored
by manganese (43). Our studies suggest that the DNA binding
activity of MTF-1 is not affected by the other transition metals
examined, and that zinc binding, at least as assessed by EMSA
of MTF-1 activity, is of a much lower affinity than might be
predicted from studies of zinc finger peptides which bind zinc with picomolar to nanomolar binding constants (15, 16, 40, 43). Therefore, MTF-1 appears to be unique among the zinc finger transcription factors in its relatively low affinity yet high specificity for zinc activation of DNA binding activity. The factors governing metal specificity and affinity of binding to MTF-1 remain to be determined.

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The DNA Binding Activity of Metal Response Element-binding Transcription Factor-1 Is Activated \textit{in Vivo} and \textit{in Vitro} by Zinc, but Not by Other Transition Metals

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