ANTIOXIDANTS & REDOX SIGNALING
Volume 3, Number 4, 2001
Mary Ann Liebert, Inc.

Forum Review

Metal Response Element (MRE)-Binding Transcription Factor-1 (MTF-1): Structure, Function, and Regulation

DAVID P. GIEDROC, XIAOHUA CHEN, and JULIUS L. APUY

ABSTRACT

Metal-responsive control of the expression of genes involved in metal metabolism and metal homeostasis allows an organism to tightly regulate the free or bioavailable concentration of beneficial metal ions, such as zinc, copper, and iron, within an acceptable range, while efficiently removing nonbeneficial or toxic metals. Emerging evidence also suggests that metal homeostasis is intimately coupled to the oxidative stress response in many cell types. The expression of genes that encode metallothioneins in all vertebrate cells is strongly induced by potentially toxic concentrations of zinc and cadmium, as well as in response to strong oxidizing agents, including hydrogen peroxide. This induction requires a *cis*-acting DNA element, termed a metal response element (MRE), and MRE-binding transcription factor-1 (MTF-1), a Cys_2-His_2 zinc finger protein. This review summarizes recent progress that has been made toward understanding the structure, function, and metalloregulation of mammalian MTF-1. Antioxid. Redox Signal. 3, 577–596.

METAL HOMEOSTASIS

METAL IONS play multiple biological roles, serving as structural components of proteins or as intrinsic cofactors in enzyme-catalyzed reactions (13). Metal ion homeostasis reflects the maintenance of the free or bioavailable concentration of essential metal ions, such as zinc, copper, and iron, at levels that are optimal to cellular metabolism and survival, coupled with the expulsion of toxic metals, *e.g.*, mercury, cadmium, lead, and arsenates, which play no biological role (49). All organisms have evolved molecular circuitry of metalloregulation that incorporates distinct control mechanisms for the homeostasis of essential metal ions, depending on whether the cell senses that there is limiting or excess free metal concentrations present intracellularly. In general, under metal-deficient conditions, the transcription of genes that encode for membrane uptake and intracellular transport proteins is activated, which enables the cell to scavenge metal efficiently from the environment and utilize it (19). Under conditions of excess metal, the expression of genes encoding specific metal export pumps (*e.g.*, P-type ATPases, which function as membrane transporters) (67) or highly specific, intracellular metal chelators that sequester metals, *e.g.*, metallothioneins (MTs) in cyanobacteria (72) and vertebrate cells (31), are two common regulatory mechanisms used to maintain the free concentration of an essential metal ion in a range compatible with cell viability. Intracellular metallochaperones that perform metal-specific transporter or enzyme ac-

Center for Advanced Biomolecular Research, 2128 TAMU, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843-2128.

tivation functions are also likely to play a major role in homeostatic mechanisms, particularly with respect to copper homeostasis (12, 58, 63).

Emerging evidence now suggests that metal homeostasis is also strongly linked to the oxidative stress response in many cells types. For example, in *S. cerevisiae*, it has been shown that treatment of cells with high concentrations of nitric oxide prevents the ability of these cells to detoxify copper, through an O_2-requiring *S*-nitrosylation of the cysteine thiolates in the Cu-activated transcription factor Ace1 (10, 65). Homozygous deletion of Cu,Zn-superoxide dismutase (SOD) in *S. cerevisiae* shows strong defects in iron homeostasis (18). With respect to zinc homeostasis, evidence has been obtained for *S*-nitrosylation of Cys thiolates in mammalian MT *in situ* upon treatment of cells with nitric oxide; this presumably results in an increased intracellular concentration of zinc (57). Oxidative stress also strongly induces the expression of mouse MT (MT-I and MT-II) genes (15); similarly, in the livers of mouse harboring a homozygous deletion of the gene encoding Cu,Zn-SOD, MT-I and MT-II expression is strongly elevated (24). Both responses require the metal response element (MRE)-binding transcription factor-1 (MTF-1), which therefore links zinc homeostasis with intracellular redox regulation (for a review, see 1).

ZINC HOMEOSTASIS

Zinc plays essential and ubiquitous catalytic and structural roles in all of biology (13). Catalytic zinc ions are present at the active sites of many hydrolytic enzymes, whereas structural zinc plays an integral tertiary structural role in many proteins, most prominently exemplified by zinc finger proteins (4, 13). Although the precise mechanism of zinc toxicity is not known, too much zinc would compete for enzyme active sites containing other redox-active metal cofactors, and/or alter homeostatic mechanisms of other metal ions by competing for metal transporter sites. Zinc is also a potent inhibitor of electron transport in bacteria and mitochondria (3). Zinc-specific metalloregulation has been documented to occur in all organisms in which it has been sought, including mammalian cells (59), plants (26), yeast (76, 77), and bacteria (54). Zinc metalloregulatory transcription factors have been cloned and partially characterized from cyanobacterial (44, 71) and other eubacterial organisms that mediate the response of cells to both zinc-deficient and zinc-excess conditions (23, 54).

MREs

In vertebrate cells, zinc homeostasis is at least partially maintained by the zinc-dependent transcriptional regulation of the expression of MT (MT-I and MT-II) genes (68, 69). MTs are small cysteine-rich proteins (51) that strongly chelate Zn(II) and Cd(II) in vertebrate cells. Targeted gene disruption of MT-I and MT-II genes in embryonic cells and transgenic mice reveals that neither gene is essential for survival, and suggests that both MTs play a role in detoxifying zinc and cadmium, as well as provide protection against oxidative stress induced by reactive oxygen species (ROS), possibly by scavenging these ROS radicals (36, 39, 43). MTs may also play a role in redox sensing and zinc mobilization as a function of the energy state of the cell (42). In addition, it has recently been shown that MT can play an important role in helping mammalian cells scavenge zinc from the environment following conditioned zinc deprivation (70). Transcriptional regulation of MT genes requires the interaction of MTF-1 (7, 28, 74) (*vide infra*) with MREs (69) situated in the promoters of zinc-inducible genes (Fig. 1).

The MRE (Fig. 1) is the *cis*-acting DNA element found in the promoters of zinc-regulated genes to which MTF-1 and other zinc-activated transcription factors, including ZRF (50), M96/ZiRF1 (64), and MEP-1, bind (for a review, see 37). The MRE constitutes an imperfectly conserved 12-base pair sequence motif (Fig. 1) and contains two subdomains, distinguished on the basis of their sequence conservation, in which a highly conserved 7-base pair 5′-TGCRCNC functional core motif is flanked by a less well conserved 5-base pair GC-rich domain, 5′-GGCCC (Fig. 1) (14). As shown in Fig. 1 for the mouse MT-I (mMT-I) and human MT-IIA (hMT-IIA) genes, like many eukaryotic en-

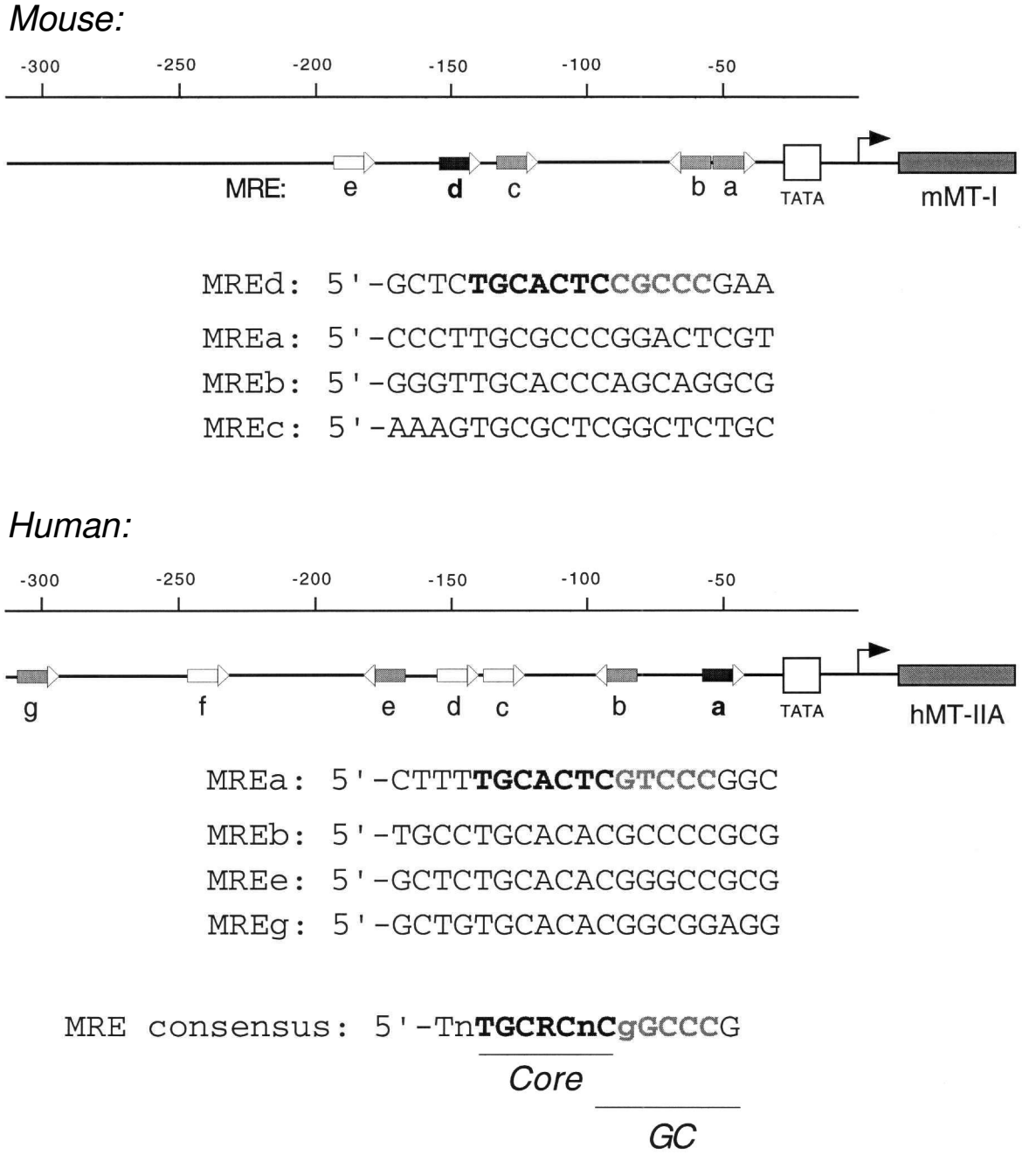


FIG. 1. Comparison of the promoter regions for the mouse MT-I (mMT-I) and human MT-IIA (hMT-IIA) genes, illustrating the positions and orientations of the MREs. MREs with the highest zinc responsiveness are represented by black arrows, whereas those with intermediate or low/no zinc inducibility are gray or white, respectively. An MRE consensus sequence is also shown. Adapted from Culotta and Hamer (14) and Koizumi *et al.* (34).

hancer sequences, MREs are often tandemly arranged in the promoters of the genes that are zinc-regulated, and found in both orientations relative to the start site of transcription. Some MREs, *e.g.*, MREd from the mMT-I gene, also contain an overlapping binding site for the general transcription factor Sp1 (74); recent studies suggest that Sp1 may play a negative regulatory role in MTF-1 mediated transcription activation (48). The MRE contains a number of 5′-CpG sequences, which are potential sites of modifications for cytosine methyltransferases. Methylation of MRE sequences leads to variable effects on MTF-1 binding activity *in vitro*, which range from no effect for a strong MRE, *e.g.*, MREd (Fig. 1), to those that abolish MTF-

1 binding to weaker MREs (61). Other studies show that the expression of mMT-I is effectively silenced by hypermethylation of MREs in solid rat hepatomas (25).

Koizumi et al. (34) determined the hMTF-1 binding activity and intrinsic zinc responsiveness of each of the seven MREs found in the hMT-IIA gene (see Fig. 1). When the results were compared with previous findings for the mMT-I gene (14), they concluded that the two most potent MREs in each promoter are highly conserved (10 of 12 base pairs are identical) and conform to a "strong MRE consensus" sequence, 5′-TGCACTC(G/C)(T/G)CCC. In addition, they noted that strong MREs often conserve a T residue two nucleotides upstream of the strong MRE and an additional GC cluster at the 3′ end of the consensus sequence. Functional characterization of a set of nonoverlapping four consecutive base pair substitution mutants of the hMREa and flanking regions revealed that, as expected, all 12 base pairs of the MRE are required for basal and zinc-activated expression in a heterologous system (34). Surprisingly, mutation of four consecutive base pairs just upstream of the 5′-TGCACTC sequence also resulted in complete loss of basal and zinc-responsive transcription; in contrast, substitution of the 4 base pairs immediately downstream of the 12-base pair consensus sequence had little or no effect (34, 35). In the pufferfish, the consensus MRE largely conforms to the MRE-s sequence (2), a consensus mammalian MRE designed by Radtke *et al.* (59), 5′-TGC**A**CACGGC**A**C.

MTF-1

MTF-1 is a constitutively expressed protein in mouse and human cells of ~80 kDa that contains six Cys_2-His_2 zinc fingers and multiple domains for transcriptional activation, the latter of which are thought to be important for interaction with the transcriptional machinery (Fig. 2). MTF-1 is an essential protein required for both basal and heavy metal-induced expression of MT-I and MT-II genes in mouse and human cells (28). In addition to regulation of the MT genes, recent work suggests that MTF-1 and MREs are required for metalloregulation of the mouse ZnT1 gene, a ubiquitously expressed plasma membrane-localized zinc efflux gene required for cell viability (38, 52), as well as the heavy chain of γ-glutamyl-cysteine synthetase, an essential enzyme in glutathione biosynthesis (27). This latter finding suggests a link between zinc homeostasis and the maintenance of an appropriate reducing potential inside cells (*vide infra*) (*cf.* 42).

MTF-1 AS A ZINC SENSOR

Numerous reports are consistent with the hypothesis that MTF-1 itself is a cytoplasmic zinc sensor that is characterized by increased nuclear MRE-binding activity upon zinc treatment *in vivo*, as evidenced by genomic footprinting experiments (15), and from *in vitro* electrophoretic mobility shift assays (EMSA) (5, 16). Consistent with this model, both zinc and cadmium appear to stimulate the nuclear translocation of MTF-1 in mouse and human cells, in a form that is activated to bind DNA *in vitro* (50, 66). Activation of DNA binding has been shown to occur in crude nuclear extracts isolated from zinc-treated versus control cells (5, 74), with crude MTF-1 preparations obtained from programmed *in vitro* transcription/translation reactions (16), or with partially purified recombinant hMTF-1 preparations (34, 35). Activation of MRE binding *in vitro* typically requires the addition of *total* Zn(II) to the binding buffers in the 50–200 μM range. MRE binding of MTF-1 is *not* activated by Cd(II), yet cadmium is a strong inducer of MT expression *in vivo* in a mechanism that requires MTF-1; this may be an indirect effect of increased concentrations of bioavailable zinc as a result of Cd(II) displacement in cells (1). Oxidative stress induced in cultured mammalian cells by H_2O_2 and *tert*-butylhydroquinone also activates MTF-I to bind to the MREs in the MT-I gene as evidenced by genomic footprinting, in a manner that appears indistinguishable from that which occurs with zinc treatment (15). Current thinking suggests that this is an indirect effect as well, with ROS causing the release of intracellular zinc by oxidation of cellular thiols, *e.g.*, glutathione, which in turn releases Zn(II) from low molecular weight stores that might include

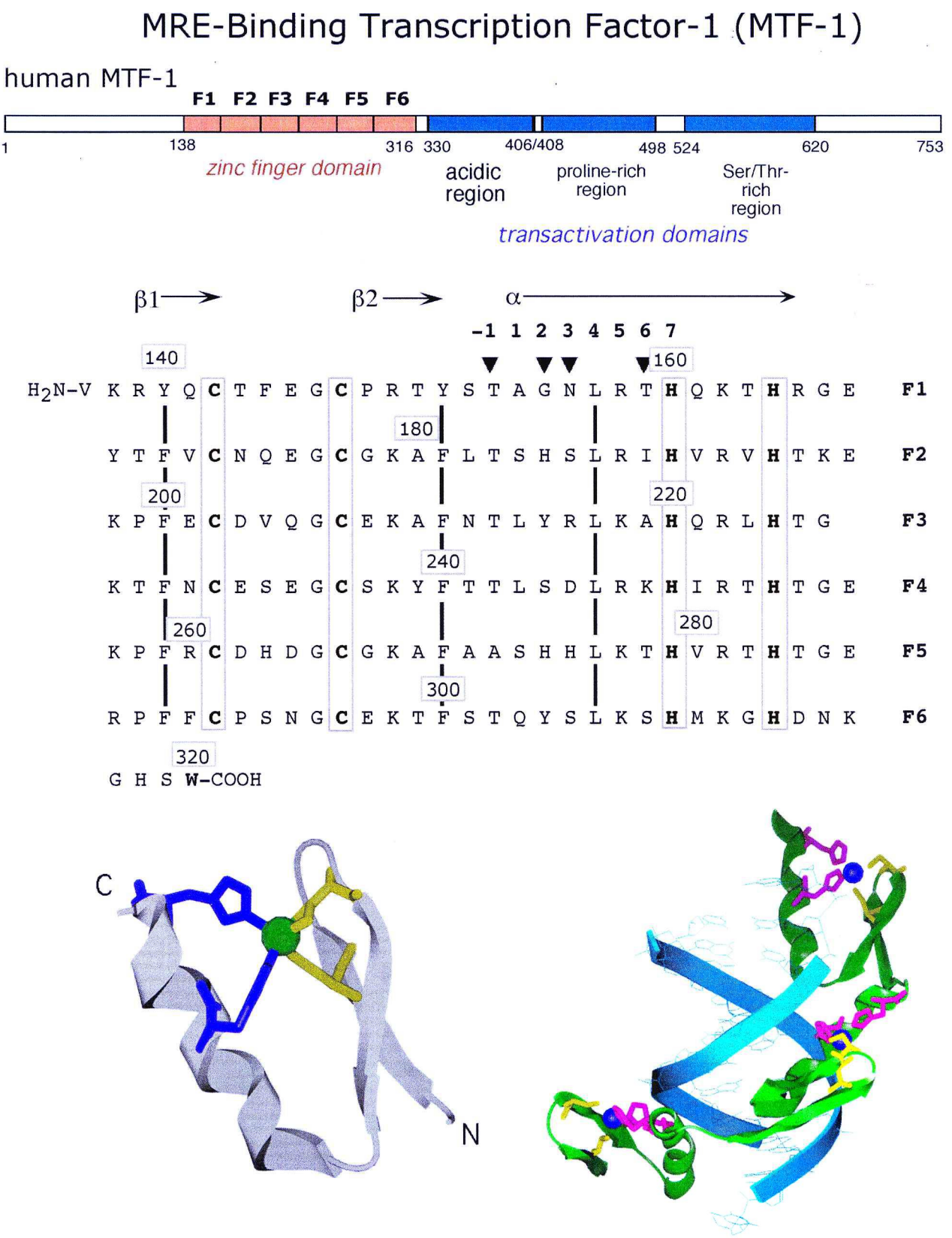


FIG. 2. The domain organization (*top*) and primary structure of the zinc finger domain (*middle*) of human MTF-1. The zinc finger domain consists of six consensus Cys_2-His_2 zinc finger motifs, which are predicted to fold into $\beta\beta\alpha$-finger structures, with the conserved Cys and His residues providing for tetrahedral coordination of a Zn(II) ion (**bottom, left**). ▼, residues in the −1, +2, +3, and +6 positions relative to the start of the α-helix in each $\beta\beta\alpha$ structure (**middle**) are commonly involved in making base-specific contacts with the major groove of the DNA (**bottom, right**) (75). Both structures are adapted from the crystallographic structure of the Zif268–DNA complex (55).

MT (1). These proposed mechanisms of regulation of gene expression by MTF-1 by zinc and oxidative stress are summarized in Fig. 3.

These studies seem to document clearly the direct, zinc-dependent activation of the MRE-binding activity of intact MTF-1, which is required for zinc inducibility of gene expression *in vivo*. This contrasts with studies of a purified, recombinant zinc finger fragment of MTF-1 expressed in *E. coli*, termed MTF-zf, in which

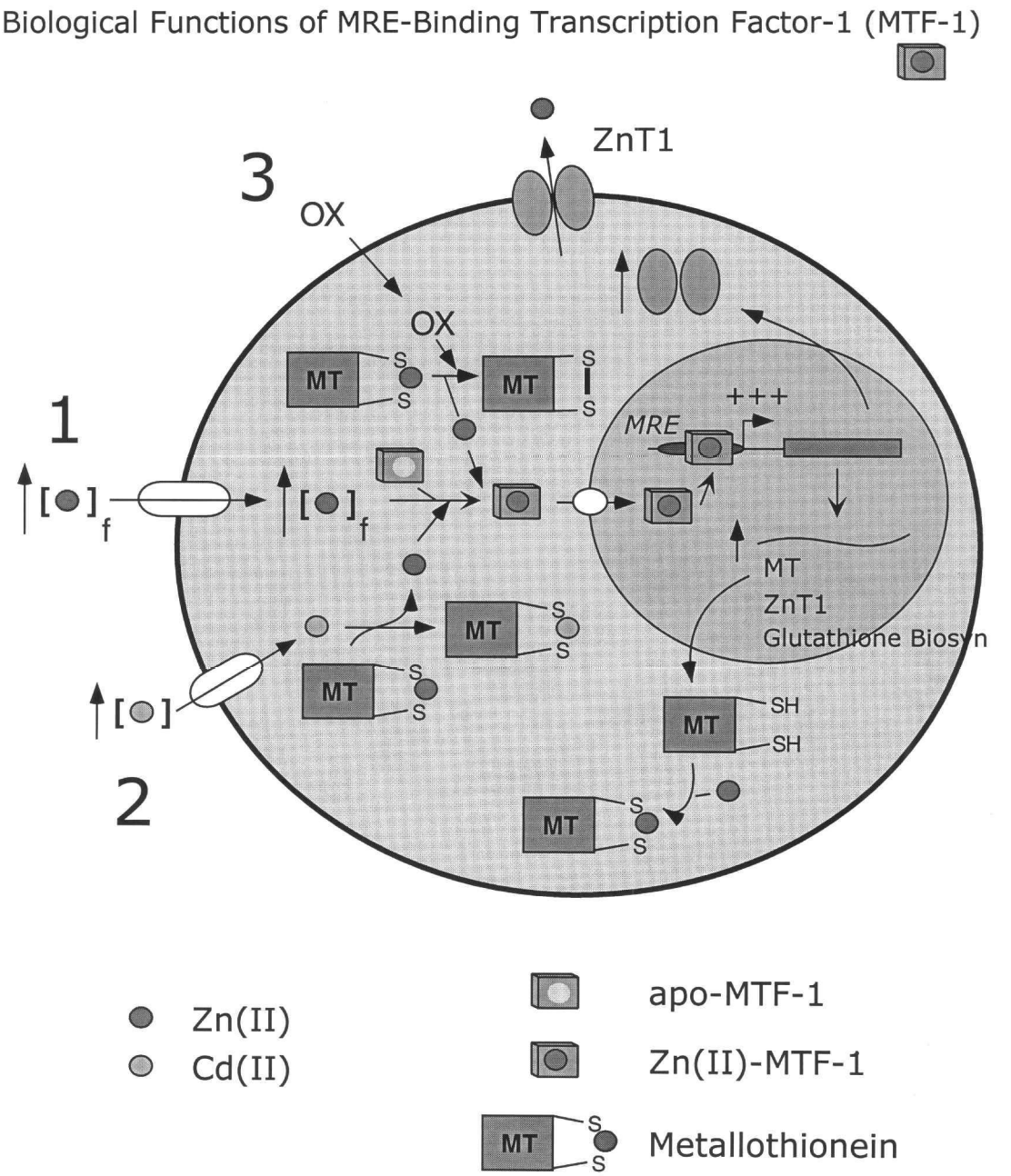


FIG. 3. Cartoon model illustrating the biological functions of MTF-1 (see text for details). The model illustrates three ways (denoted **1, 2,** and **3**) in which MTF-1 can be activated to bind Zn(II), translocate to the nucleus, and activate the expression of genes that contain MREs. Activation of MTF-1 DNA binding and transcriptional activity by Cd(II) (2) or oxidative stress (3, OX) in this model is proposed to be an indirect effect of the release of zinc bound to low molecular weight cytoplasmic chelators (MTs and/or glutathione) upon Cd binding or oxidation of metal-coordinating thiolates, respectively.

it has not yet been possible to demonstrate clearly the reversible zinc activation of MRE binding by MTF-zf *in vitro* (8). The origin of the discrepancy is unknown, but suggests that flanking and/or activation domains in some way contribute to maximal zinc activation of DNA binding (*cf.* 35) and MT gene transcription by MTF-1. In any case, oxidation of even a subset of the zinc fingers of MTF-zf weakens DNA binding affinity of MTF-zf significantly; thus, direct oxidation of Cys residues of the zinc finger domain of MTF-1 is unlikely to result in reversible *activation* of the MRE-binding affinity (8). Interestingly, it has been shown that the MRE-binding activity of MTF-1 in crude nuclear extracts can be modulated by the redox conditions of the binding buffer, with the absence of dithiothreitol (a reducing agent) promoting apparently constitutive binding to the MRE *in vitro* (35). The molecular basis for this effect is unknown, and may well involve the C-terminal Cys cluster in the protein (*vide infra*) (Fig. 4).

MTF-1: PRIMARY AND DOMAIN STRUCTURE

The gene encoding MTF-1 has been cloned from the mouse (58), human (7), the Japanese pufferfish *Fugu rubripes* (2), and *Drosophila melanogaster*; in addition, a cDNA encoding a single amino acid substitution variant of hMTF-1, termed hMTF-1b (formerly ZRF; $His^{185} \rightarrow Tyr$), has also been characterized (50). The overall organization of the protein-encoding regions of these genes and a comparison of their primary structures are shown in Figs. 2 and 4, respectively. There is a DNA binding domain characterized by six contiguous, canonical Cys_2-His_2 zinc finger domains toward the N-terminus of the protein, and at least three transcriptional activation domains, termed acidic-rich, proline-rich, and serine/threonine-rich domains, respectively (Figs. 2 and 4). Each of these domains in the mouse and human MTF-1 proteins confers some degree of transcriptional activation activity when fused to a heterologous DNA binding domain (60). All proteins also contain an N-terminal domain, currently of unknown function (Fig. 4). In addition, the mouse (and rat) sequence is truncated at the C-terminus relative to the human, pufferfish, and *Drosophila* sequences (2).

As the proteins encoded by the human and mouse genes are so highly conserved (92.4% and 95.5% amino acid identity and similarity, respectively), comparison of these sequences with the pufferfish sequence allows a more extensive comparison over 400 million years of evolution (Fig. 4) (2). The *Fugu* zinc finger domain is 90.5% identical (~94% similarity; nearly 80% identity at the nucleotide level) to the mouse and human proteins; this represents the most highly conserved region of the molecule (Fig. 4). Consistent with this, the MRE sequences are also highly conserved among all three species (see above). Overall, the *Fugu* amino acid sequence exhibits 57% identity and ~72% similarity to the two mammalian sequences. In contrast, the only region of significant homology between *Drosophila* and mammalian/fish MTF-1 is in the zinc finger domain (Fig. 4). Other conserved domains between the mammalian and pufferfish sequences include a ~53-amino acid region immediately preceding the zinc finger domain, which has been speculated to contain a nuclear localization signal (KRKEVKR), as well as potential sites for phosphorylation in the vicinity of this putative nuclear localization signal (66).

Another region of striking conservation is a 32-amino acid stretch of largely hydrophobic amino acids that immediately follow the Ser/Thr-rich domain. This run of amino acids contains a hydrophilic ^{629}Glu-Glu-Gln-Cys-Gln-Cys-Gln-Cys-Ala-Cys-Arg-Asp^{640} sequence that is rich in potential zinc-liganding amino acids. Interestingly, although this exact sequence is not conserved in *Drosophila* MTF-1, there is an analogous ^{547}Cys-Asn-Cys-Thr-Asn-Cys-Lys-Cys-Asp-X_4-Cys-His-X_2-Asp-Cys^{565} sequence, followed by 16 additional Cys residues that are clustered in pairs and extend nearly to the C-terminus of the protein (residue 791). The functional importance of these conserved Cys residues is unknown and has not been directly tested in any MTF-1. However, it is tempting to speculate that they may be involved in zinc recruitment, exchange, or insertion processes that might function in metal and redox homeostasis, particularly under condi-

tions of extreme free zinc limitation, in a manner analogous to that proposed for the Cys pair in domain III of the Cu(I) chaperone of human Cu,Zn-SOD1, hCCS (62, 63).

MRE-binding activity which is immunologically similar to mammalian MTF-1 has also been identified from cell lines derived from zebrafish and trout with one isoform found in zebrafish, and two (MTF-1H and MTF-1L) in trout cells (17). The gene(s) encoding these MTF-1 proteins have not yet been cloned.

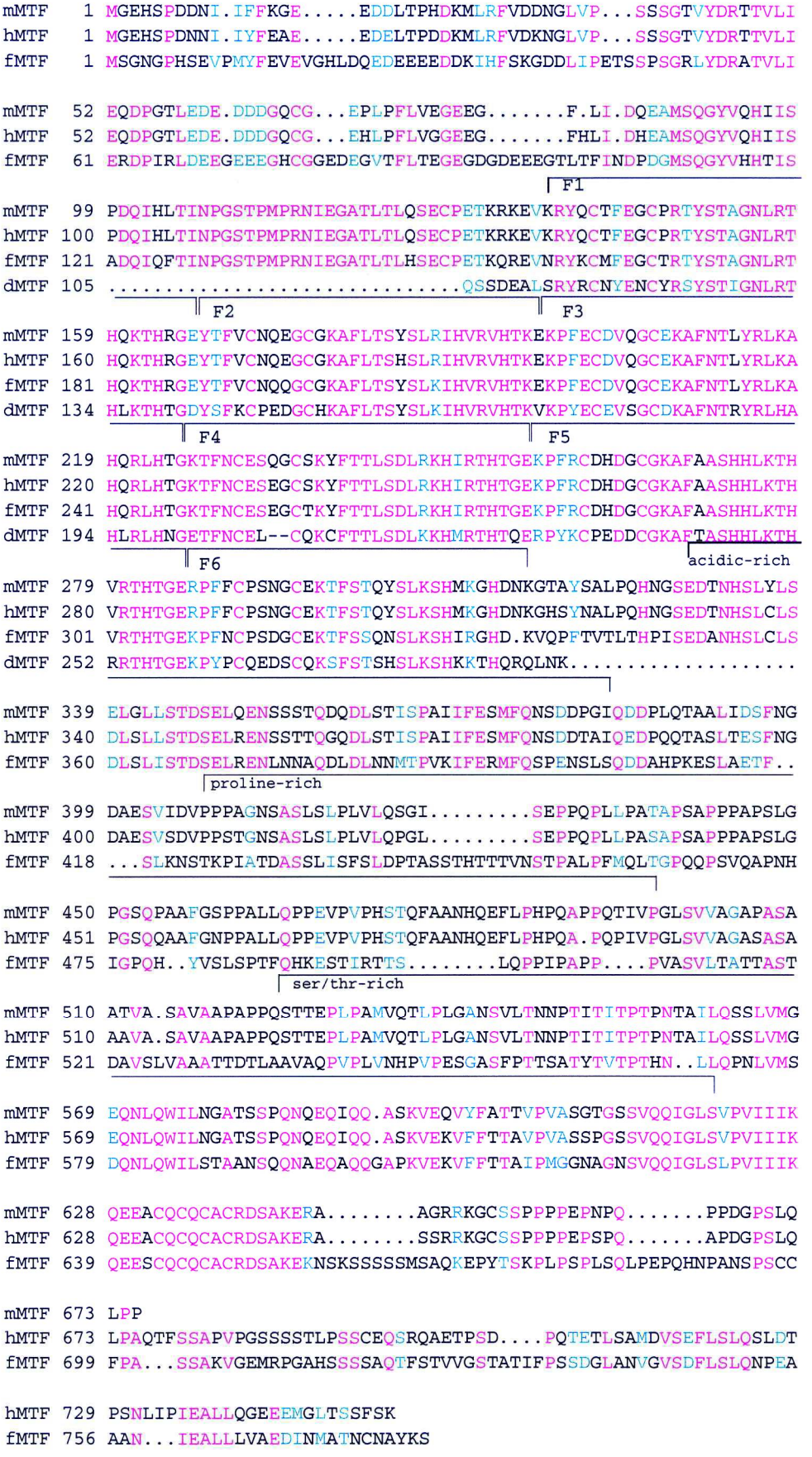
THE ZINC FINGER DOMAIN OF MTF-1

All MTF-1 genes encode a protein characterized by six canonical Cys_2-His_2 zinc finger motifs that conform to the general consensus sequence (Y/F)-X-C-X_4-C-X_3-(Y/F)-X_5-L-X_2-H-X_3-H, where X is any amino acid (Fig. 2). Each of these sequences is connected by a four- or five-residue linker, which itself is also rather highly conserved. It was projected that these sequences were very likely to fold in the presence of Zn(II) to form a well structured $\beta\beta\alpha$ domain (Fig. 2). In the $\beta\beta\alpha$ domain, the Zn(II) ion forms a tetrahedral coordination complex in which the two conserved Cys residues from the two-stranded antiparallel β-sheet and two His residues toward the C-terminus of the α-helix provide the ligands to the metal ion (40; for a review, 75). The conserved aromatic and hydrophobic amino acids form a minihydrophobic core that sits "atop" the finger structure. The His-X-(Arg/Lys)-X-His sequence, which characterizes all putative zinc fingers in MTF-1, may adopt regions of 3_{10} helix in order to accommodate zinc binding (75).

The putative finger domains of MTF-1 are connected by nearly consensus TG(E/Q)(K/R)P linkers, with the exception of linker between finger domain 3 (F3) and F4, which is one residue shorter, TGKT. Examination of the conformations that the TGEKP and other linkers adopt in protein–DNA complexes of known structure reveals that the Gly plays the most critical role in completing the C-cap structure on the α-helix, whereas the Glu residue in the 3-position often orients the Arg in His-X-(Arg/Lys)-X-His sequence of the preceding finger to make phosphodiester backbone contacts (75). The Lys residue in the 4-position in the linker often makes a direct or water-mediated hydrogen bond to the phosphate backbone; each of these interactions is expected to provide electrostatic stabilization to the complex. Although extensive data are limited, linkers shorter than five amino acids will tend to pull finger domains closer to one another on the DNA, and in at least one case [*Xenopus* transcription factor IIIA (TFIIIA)], has been shown to cause adjacent fingers to bind to consecutive major and minor grooves along the DNA (47).

Much is known about how Cys_2-His_2 zinc finger domains bind to specific DNA sequences (75). The crystallographic structure of the mouse transcription factor Zif268 bound to DNA is accepted as the prototypical example of how Cys_2-His_2 proteins bind to specific DNA sequences (20, 55). Basically, the three α-helices derived from each of the zinc finger domains of Zif268 fit into the major groove, with the binding of successive fingers causing the protein to wrap around the DNA (Fig. 2). Each finger domain interacts with consecutive, overlapping 4-base pair subsites, with the majority of base specific contacts made to three contiguous base pairs, but involving primarily one strand of the DNA (termed the primary strand). The three fingers of Zif268 are oriented so that finger domain F1 is at the 3′-end of the primary strand, with F3 at the 5′-end of the primary strand (see Fig. 6 below). In each of these canonical fingers, the amino acid side chains in the −1, +3, and +6 positions relative to the start of the α-helix form specific hydrogen bonds with the nucleotide bases in the 3′, middle, and 5′ base of the primary strand that

FIG. 4. Amino acid sequence alignment of MTF-1 from mouse (59), human (7), *Fugu* (2), and *Drosophila* (B. Zhang, D. Egli, O. Georgiev, and W. Shaffner, unpublished observations; EMBL accession no. AJ297844). Identical residues are shown in pink, similar residues in green, and nonconservative substitutions in black. Only the zinc finger region of the *Drosophila* sequence is shown because there are no other regions of significant sequence homology with the mammalian/pufferfish proteins. Adapted from Auf der Maur *et al.* (2).


F1
F2
F3
F4
F5
F6
acidic-rich
proline-rich
ser/thr-rich

makes up each 3-base pair subsite, respectively (Fig. 2). The only significant contact to the complementary strand in these canonical fingers is a hydrogen bond from the side chain in the +2 position to the nucleotide just 5′ to the 3-base pair triplet on the complementary strand (see Fig. 6 below).

More recent NMR and crystallographic structures have revealed that canonical fingers often contain Arg or Lys in the −1 and/or +6 positions of the DNA binding α-helices, which nearly always interact with guanosines in the 3′ and 5′ base of the base triplet, respectively, on the primary strand (21, 22, 29, 32, 47, 56, 75). When these residues are not present, as they are with only one exception (F4) in MTF-1, it becomes difficult to predict how this multifinger protein might interact with the MRE (see below). However, several of these structures do reveal a wealth of alternative DNA recognition patterns made by what are now known as "nonstandard" fingers, many of which have more extensive interactions with the complementary strand than present in the Zif268-DNA complex, as well as unusual interactions involving amino acids in the −1 and +2 positions (11, 75).

Simple extension of the structure of the Zif268 or the N-terminal three fingers of TFIIIA (F1–F3), for example, bound to their cognate DNA recognition sequences predicts that the six zinc fingers of the MTF-1, if they were all to fold up into zinc finger structures, would predict a specific interaction site of ~18–20 base pairs, considerably larger than the conserved 12-base pair MRE sequence. This fueled speculation that not all fingers of MTF-1 were required to interact specifically with the MRE, because the 12-base pair site is only of sufficient length to accommodate three or four contiguous fingers (8, 61). The hypothesis was made that a subset of fingers in MTF-1 could perform a structural role in interacting strongly and constitutively with the MRE, with the remaining fingers performing some other role, *e.g.*, a metalloregulatory role, or in mediating intra- or intermolecular protein–protein interactions important for zinc-dependent activation of gene expression. Consistent with this, it has been shown that activation of the MRE-binding activity of MTF-1 by 30–100 μM added zinc required the zinc finger domain, although flanking regions often appear to enhance the extent of activation (6, 16, 35).

Another prediction of this hypothesis was that a subset of fingers would exhibit high affinity for zinc and that these would specify the "structural" fingers of the molecule, with remaining fingers characterized by a far lower affinity for the metal; these, in turn might be regulatory. To test these ideas, Chen *et al.* (8) showed that a bacterially expressed recombinant form of the zinc finger fragment of hMTF-1 could be obtained that contained six equivalents of bound zinc, under conditions where all 12 Cys in the molecule are in their reduced and potentially metal-liganding state. This protein, termed Zn_6 MTF-zf, was shown to bind tightly ($K_a = 3 \times 10^8\ M^{-1}$ at 0.20 M NaCl, pH 7.0, 25°C) and specifically to form a 1:1 complex with a coumarin-labeled oligonucleotide containing a single mouse MREd sequence. The binding was shown to be strongly salt-dependent, with as many as five Na^+ ions released upon binding, and exhibited a complex temperature dependence characterized by a large negative ΔC_p° of binding (-1.6 kcal mol^{-1} K^{-1}), with a temperature of maximum stability of the MTF-zf-MREd complex of ~18°C. Perhaps more importantly, these studies demonstrated that the zinc finger domains within MTF-zf were structurally heterogeneous, with a subset of the finger domains (2–3) characterized by weak zinc binding affinity [these Zn(II) ions were readily lost upon extensive anaerobic dialysis or via chelation with the low-affinity zinc chelator, Mag-Fura-2], with three or four sites of relatively higher affinity (8).

Circular dichroism spectroscopy (9) and more recent heteronuclear NMR studies of the MTF-zf fragments reveal that finger domains F1–F4, as well as F6, are intrinsically capable of adopting the predicted $\beta\beta\alpha$-structure in the presence of Zn(II). In contrast, F5, at least in the absence of DNA, appears to fold into one or more alternative structures at excess Zn(II), with the $\beta\beta\alpha$ conformation only observed as a stable folding intermediate in a Zn(II) titration experiment as monitored by a ^{1}H-^{15}N correlation spectroscopy (D. Giedroc, X. Chen, M. Pennella, and A. Li-Wang, submitted for publication).

Figure 5 shows the results of a pulsed alky-

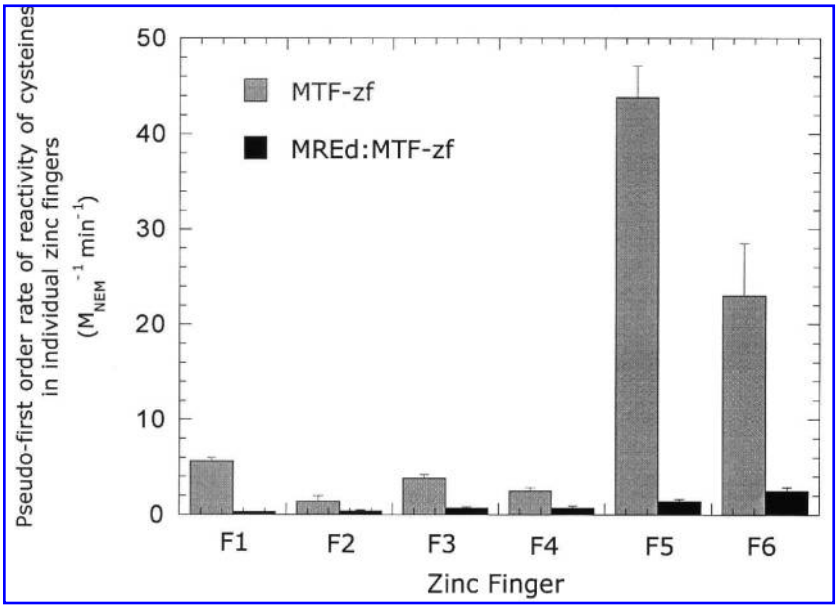


FIG. 5. Plot of pseudo-first-order rates of reactivity of pairs of cysteine thiolates in individual zinc finger domains of Zn_6 MTF-zf toward alkylation by NEM as determined by ratiometric pulsed-alkylation mass spectrometry. Blue columns, free MTF-zf; red columns, MTF-zf-MRE complex. The kinetics of pulsed alkylation of cysteine thiolates were determined by exposing Zn_6 MTF-zf to a pulse of variable time duration with perdeuterated d_5-NEM, followed by a chase with excess protonated H_5-NEM to fully derivatize the protein. Following complete proteolysis by trypsin or chymotrypsin, the extent of derivatization by d_5- and H_5-NEM was determined by MALDI-TOF mass spectrometry (J. Apuy, X. Chen, T. Baldwin, D. Russell, and D. Giedroc, submitted for publication).

lation mass spectrometry experiment that was used to rank order the relative stabilities of individual zinc chelates in Zn_6 MTF-zf, as assessed by the reactivities of individual Cys-X_4-Cys sequences to the sulfhydryl-specific alkylating reagent *N*-ethlymaleimide (NEM) (J. Apuy, X. Chen, T. Baldwin, D. Russell and D. Giedroc, submitted for publication). These data establish that the order of reactivity is F5 $\approx$ F6 $>>$ F1 $>$ F2 $\approx$ F3 $\approx$ F4, and strongly implicate F5 and F6 as the weak zinc-binding fingers of the molecule. Interestingly, probing of the MTF-zf-MREd complex by limited proteolysis reveals that these C-terminal finger domains are digested to smaller fragments, with a major tryptic site at Arg^{260}, just C-terminal to the linker that connects F4 and F5 (9). Finger domains F1–F4 when bound to the MREd are strongly protected from further proteolysis. These results implicate finger domains F1–F4 as intimately associated with the MREd, with C-terminal domains F5 and F6, less strongly bound. This picture is largely consistent with characterization of a limited set of missing and broken-finger mutants of MTF-zf (9). F4 is clearly a structural finger. In contrast, C-terminal fingers F5 and F6 appear to make only a small, but measurable, contribution to the affinity and specificity of complex formation with the tight-binding MREd sequence. In the absence of finger domains F1–F3, a fragment corresponding to Zn_3 F4–F6 (MTF-zf46) has no affinity for the DNA (9).

STRUCTURAL MODEL OF THE MTF-zf-MREd COMPLEX

Circular dichroism spectroscopy of the MTF-zf-MREd complex suggests that although the MRE is essentially B-form, MTF-zf binding induces significant A-like conformational features (9), like that previously observed for the Zif268-DNA complex (20). These conformational features may be reporting on a major groove that is wider and deeper than normal, in order to accommodate one or more α-helical recognition helices derived from the $\beta\beta\alpha$ structures (46). Fluorescence resonance energy transfer (FRET) experiments have been used to define the global orientation of the multifinger MTF-zf on the MREd oligonucleotide. These studies are most

consistent with the N-terminal finger domains F1–F4 as bound at or over the 5′-TGCRCNC sequence with the F5 and F6 domains closer to the 3′-GC-rich subdomain (9).

A detailed structural model of the interaction of finger domains F1–F4 of MTF-1, which incorporates the results from limited trypsinolysis and FRET experiments, as well as extensive crystallographic studies of other Cys_2-His_2 zinc fingers bound to their cognate recognition elements, is presented in Fig. 6. As explained below, the model of the structure is compatible with sequence conservation of the MRE (Fig. 2), as well as the amino acid sequence of the zinc finger domain from humans to pufferfish and *Drosophila* (Fig. 4). Inspection of the primary structure of MTF-1 reveals, with the exception of F4, a paucity of canonical recognition determinants in the −1 and +6 positions; thus, most of the finger domains in MTF-1 probably adopt "nonstandard" finger-DNA contacts (75).

F4, the most "standard" of the MTF-1 fingers, possesses Ser, Asp, and Lys residues in the +2, +3, and +6 positions, respectively, which taken together are predictive for a 5′-GCNG sequence on the primary strand. The only place that this occurs in the 12-base pair MRE is in nucleotides G10′ to G7′, overlapping the two subdomains of the recognition element. With F4 positioned here, the Tyr and Arg residues in the +2 and +3 positions of finger domain F3 are predicted to interact with C5 on the complementary strand and G7′ on the primary strand, respectively, exactly like that which occurs in the GLI F2 and TFIIIA F5 zinc fingers, respectively (Fig. 6) (47, 56). In this arrangement, C8-G8′ base pair is "shared" by the F3 and F4 finger domains; this would appear to be compatible with the shortened linker that connects these two fingers, as well as the lack of a strong recognition determinant (*i.e.*, an Arg or Lys in the +6 and −1 positions of F3 and F4, respectively) to this base pair. With F4 and F3 positioned here, there is strong recognition determinant for the Ser in the +3 position in F2 to form a hydrogen bond with T or C in the 4′ position of the primary strand; this is consistent with the conservation of a purine in the 4 position of the core TGC**R**CNC recognition element. This single contact could well be supported by other cross-strand interactions: from Thr in the −1 position to T1 [analogous to the Leu(−1)-thymidine van der Waals contact in TFIIIA-F5] (47), and an Arg in the +5 position to G2 (as GLI-F5) (56). In addition, the model readily accommodates a Tyr substitution in the +2 position (as in the *Fugu*, mouse, and *Drosophila* proteins) and Lys in the +5 position (*Fugu*, *Drosophila*), without loss of specific interactions. Finally, Asn in the +3 position of F1 provides a strong recognition determinant for A1′, with no other obvious candidates for specific recognition of this putative F1-base pair triplet.

If one accepts the positions of F1–F4, and extends specific α-helix–major groove interactions to the F5 and F6 domains, the His residues in the +2 and +3 positions of F5 might provide specificity determinants for C12 and G12′, respectively, just like that which occurs in TFIIIA-F2 (47). Likewise, the MREd sequence contains four A-T base pairs, which are one base pair removed from the C12-G′12 base pair; here structural studies make the prediction that Thr and Ser in −1 and 3 positions might interact with consecutive thymine bases on the primary strand, like that sometimes observed in phage display experiments (75). These proposed interactions might provide an explanation for how downstream weak zinc-binding fingers in MTF-zf enhance the specificity of the high-affinity MTF-zf-MREd complex (9). However, it must be pointed out that there are no existing crystallographic structures of naturally occurring or designed zinc finger–DNA complexes where more than four contiguous zinc fingers were found to interact with the major groove, particularly those finger domains connected by TGEKP linkers, like that found in MTF-1 (75).

This structural model also accommodates the two adenosine substitutions in the consensus MRE-s sequence designed by Radtke *et al*. (59), 5′-TGC**A**CACGGC**A**C, because neither base pair is predicted to make specific interactions in the model. However, these substitutions would place thymines in the 3′ base position of two proposed base triplets in the primary strand, opposite a Thr and an Ala residue in the −1 positions of F3 and F5, respectively; the methyl groups of these residues could make

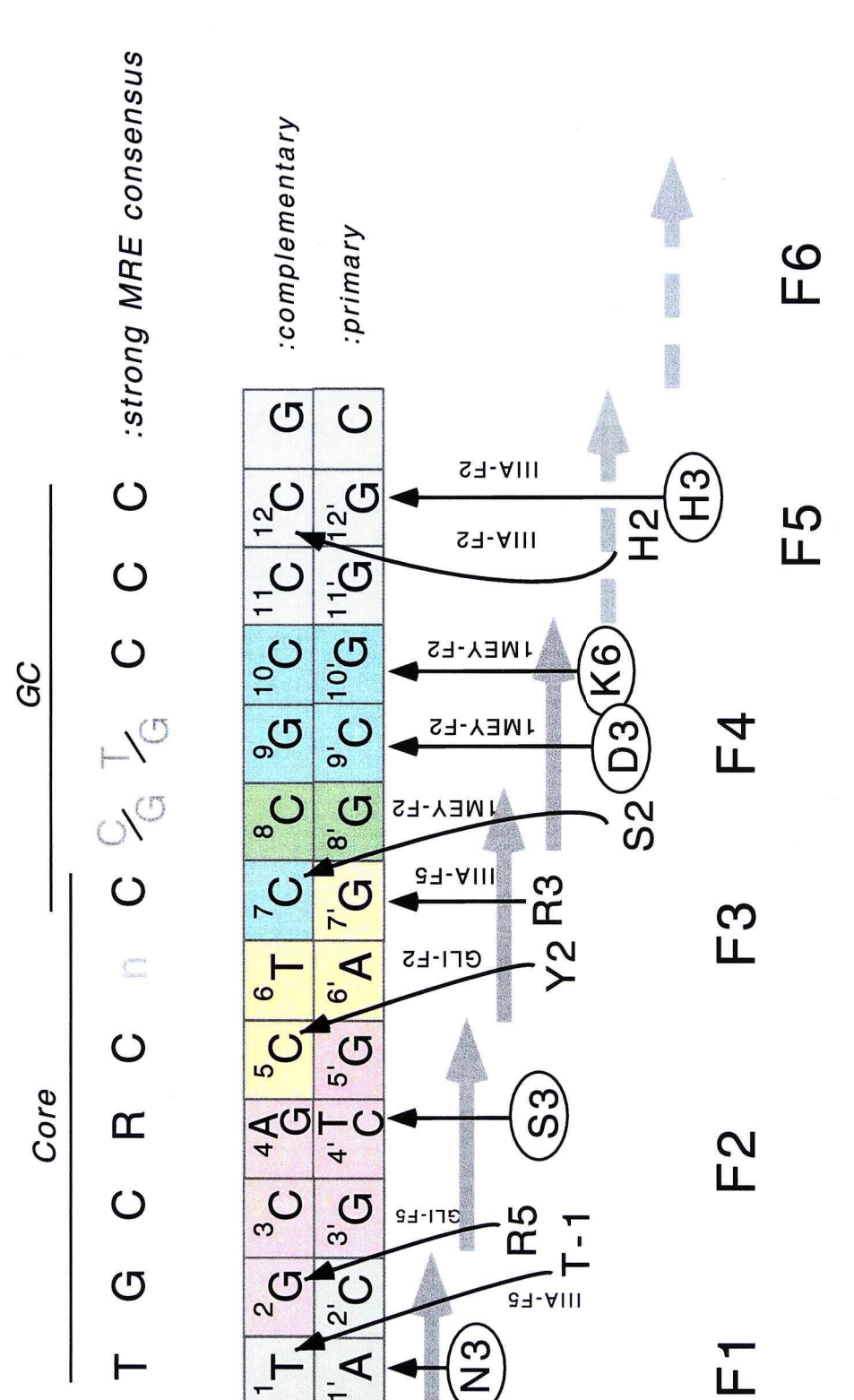


FIG. 6. Hypothetical schematic model for the interaction of the zinc finger domain of MTF-1 with the MREd sequence. Each gray arrow represents the recognition α-helix for each of the six zinc fingers of MTF-1. Overlapping 3–4 base pair subsites are color-coded. Note that the base pair subsites assigned to F3 and F4 share an overlapping C8–G8′ base pair, which is therefore shaded green. Predicted contacts to the primary DNA strand are indicated with straight arrows, and those cross-strand interactions are indicated by the curved arrows. Amino acids from the −1, +2, +3, +5, and +6 positions from individual helices that are modeled to make base-specific contacts are indicated, *e.g.*, Lys in the +6 position in F4 (K249 in Fig. 2) is designated K6 in the figure. Encircled recognition determinants are often found in naturally occurring and evolved zinc fingers with novel specificities (see ref. 75 for a review). Other predicted base-specific contacts found in structurally characterized "nonstandard" fingers are also shown, with the finger domains from which they were derived indicated. IIIA, transcription factor IIIA (47); GLI (56); 1MEY, designed zinc finger-DNA complex (32). Adapted from Choo and Schwabe (11).

van der Waals interactions with the Thy 5-CH_3 substituent (75).

It must be emphasized that this model represents only a starting point for further structural and functional studies, and has largely been constructed to maximize potential base–protein side chain interactions upon inspection of existing zinc finger–DNA structures, essentially one triplet at a time, without attempting to model the structure in detail. It must be borne in mind that attempts to optimize the specificity of individual finger-triplet interactions within a multi (3)-finger protein reveal that that precise context of finger–triplet interactions may play a nonnegligible role in specificity (30, 75). However, it is encouraging that overlapping DNA subsites in the model appear to specify potentially fully compatible interactions with neighboring zinc finger domains, with 8 of 11 strongly conserved base pairs of the consensus 12-base pair MRE sequence readily rationalized by this structural model.

MECHANISM OF ZINC METALLOREGULATION OF GENE EXPRESSION BY MTF-1

Most studies are consistent with a model that suggests that direct, reversible activation of MRE-binding affinity of MTF-1 by zinc is necessary for activation of gene expression from MRE-containing promoter sequences. The mechanism of zinc activation is at present unknown, although some progress has been made in this area. The physicochemical studies carried out with the purified zinc finger fragment of MTF-1 clearly document a structural and functional heterogeneity in the zinc finger domains of MTF-1, with finger domains F1–F4 providing much of the MRE-binding energy, with the C-terminal fingers F5 and F6 playing some other role (9). On a tight-binding MREd sequence, it was shown that the C-terminal finger domains stabilize protein–DNA complex formation, although they were not required for tight binding. In the absence of other data, these studies suggest that the C-terminal fingers F5 and F6 have zinc-binding properties that are consistent with these finger domains playing a metalloregulatory role (Fig. 7A). This is a particularly attractive hypothesis, because it would appear that only these C-terminal fingers could be reversibly activated by the addition of total Zn(II) in the 30–100 μM range. Unfortunately, to our knowledge, it has not yet been possible to conclusively test this idea directly with purified missing-finger and broken-finger mutants of intact MTF-1 *in vitro*. In addition, the model shown in Fig. 7A implies that Zn(II) may only modestly increase the MRE-binding activity of intact MTF-1, a result not in accord with most DNA-binding data derived from EMSA experiments (6, 35).

In contrast to this prediction, Bittel *et al*. (6) present evidence in support of a model in which zinc finger F1, rather than the F5 and F6 fingers, functions as the sensor finger of MTF-1 (Fig. 7B). In brief, characterization of a series of missing-finger deletion mutants of MTF-1 suggested that deletion of F1 (ΔF1 MTF-1) resulted in a protein that appeared significantly more "activated" to bind the MRE *in vitro* in an EMSA experiment, and was only partially activated by additional zinc. Limited evidence suggested that MTF-1 $-/-$ dko7 knockout cells that transiently expressed ΔF1 MTF-1 resulted in constitutive activation of the expression of a heterologous reporter gene that was insensitive to the addition of zinc to the growth medium. In contrast, deletion of F5 and F6 domains was found to have only a small effect on the zinc inducibility *in vivo* or zinc activation of DNA binding *in vitro* (6). Remarkably, characterization of a chimeric MTF-1 in which MTF-1 zinc fingers F2–F6 were replaced with the three zinc fingers of the general transcription factor Sp1 (to create F1-MSM) appeared to lead to some zinc-activated binding to an oligonucleotide containing an Sp1 binding site. Another Sp1-MTF-1 chimera termed SMS, in which the zinc finger domain of Sp1 was replaced with the zinc finger domain of MTF-1, was also shown to be activated to bind DNA by zinc *in vitro*. Unfortunately, neither chimera was shown to be zinc-inducible *in vivo*. The origin of the discrepancy between zinc activation of MRE binding *in vitro* using crude nuclear extracts and *in vivo* zinc activation of gene expression is unknown. However, these findings

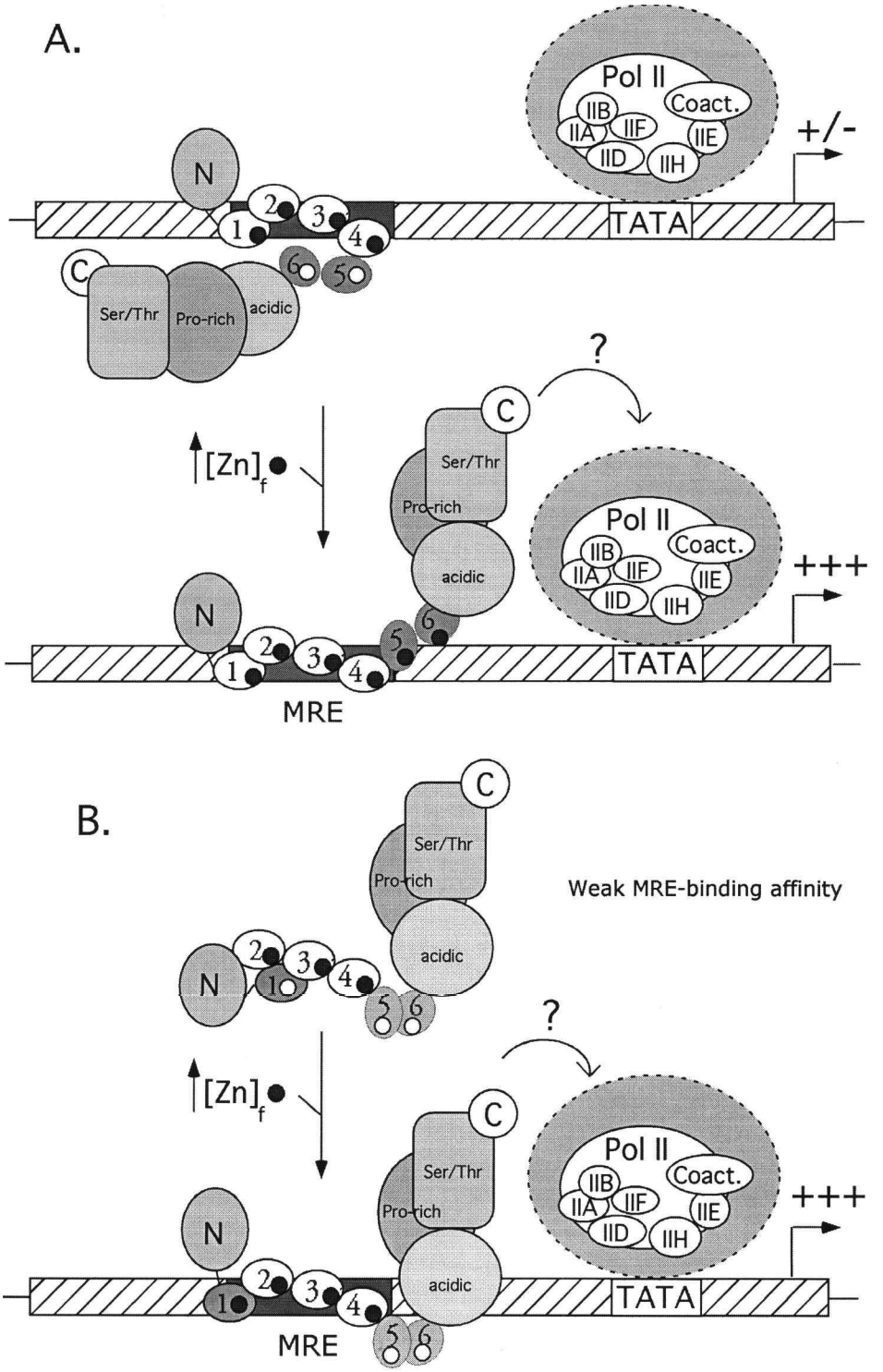


FIG. 7. Two cartoon models of how zinc binding to distinct finger domains in MTF-1 might allosterically regulate the MRE-binding and transcriptional activation activity of MTF-1. (**A**) Zn(II) binding to the weak zinc binding domains of MTF-1 (F5 and F6) causes intramolecular allosteric activation of MTF-1. This model is consistent with the physicochemical characteristics of the isolated zinc finger domain of MTF-1 (8, 9), but inconsistent with *in vivo* functional data from transient transfection experiments (6, 35). (**B**) Reversible binding of Zn(II) to F1 removes an allosteric block that prevents the strong DNA-binding fingers of MTF-1 (F2–F4) from interacting with the MRE (6). The roles of F5 and F6 in the model and whether or not they are loaded with zinc are unclear. See text for details.

are generally consistent with previous domain deletion and heterologous expression studies from the Schaffner laboratory, which were unsuccessful in clearly mapping a zinc metalloregulatory domain of MTF-1 (60). Thus, activation of DNA binding by zinc appears to be necessary, but not sufficient, for zinc induction of gene expression *in vivo*.

In any case, the results of Bittel *et al*. (6) are consistent with a model in which metal-free F1, perhaps in conjunction with the immediately adjacent N-terminal domain (35), directly negatively regulates MRE binding by the DNA binding fingers F2–F4 (and of the Sp1 fingers in the F1-MSM chimeric protein) (Fig. 7B). Only when this finger is loaded with Zn(II) are the specific DNA binding zinc fingers of MTF-1 (or Sp1 in the F1-MSM chimera) capable of strongly interacting with their DNA sequences. This model makes the testable prediction that, in contrast to the apparent behavior of the ΔF1 MTF-1 mutant (6), a broken-finger mutant in F1 (9, 35) would be constitutively inactive and *not* capable of being activated by zinc; this critical experiment has not yet been carried out. Interestingly, in our structural model, F1 is predicted to make only one base-specific contact with the MRE (Fig. 6).

We have carried out preliminary experiments in *S. cerevisiae* similar to those reported (6); these studies show that introduction of a single liganding His → Asn substitution in finger domains F1, F2, F3, or F4 completely abrogates MTF-1-dependent transcriptional activation *in vivo*, with broken-finger and missing-finger deletion mutants of F5 and F6 less functionally compromised. Current work is aimed at determining whether this represents a specific zinc response in these cells (X. Chen, D. Eide, and D. Giedroc, unpublished observations).

FUTURE EXPERIMENTS

Much remains to be learned concerning the structural biology and mechanism of action of MTF-1. Now that we have a reasonable understanding of which finger domains are required for high-affinity binding to the MRE, crystallographic and NMR structural experiments can now be rationally designed and undertaken. Although interesting in and of themselves, if successful, they will greatly extend our understanding of how nonstandard Cys_2-His_2 zinc fingers bind specifically to DNA. The availability of a preparation of highly purified intact MTF-1 into which broken-finger and missing-finger mutations can be easily introduced and functionally characterized is also needed. These MTF-1 preparations could be used for *in vitro* DNA-binding studies as well as experiments that reconstitute transcriptional activation *in vitro* in efforts to understand more fully how reversible binding of zinc by MTF-1 activates expression of MT and other genes that harbor MRE sequences.

Finally, what is the bioavailable or "free" concentration of zinc in mammalian cells in the resting state, and over what concentration range do intracellular pool(s) of zinc change within or between intracellular organelles when cells are challenged with 10–100 μM *total* zinc in the growth medium? The answer to this is not known with certainty in any cell type, although in mammalian cells, *total* intracellular zinc concentrations appear to be remarkably constant over a very wide range of extracellular zinc concentration (70). In addition, membrane-bound vesicles can be stained with fluorescent zinc-specific chelators, with essentially little or no staining in the cytosol (45, 53). These pools of vesicular zinc may correspond to the mammalian late endosome (33) and might be functionally analogous to those contained within the yeast vacuole, an organelle known to play a role in zinc storage and zinc cycling and homeostasis in *S. cerevisiae* (41). Perhaps more importantly, these studies suggest that "free" zinc concentration in the cytoplasm of higher eukaryotes may well be vanishingly small. If this is the case, then this would in fact argue *against* a significant functional role for weak zinc-binding fingers F5 and F6 in MTF-1 in metal sensing; this is consistent with available *in vivo* data (6).

Interestingly, studies of the cyanobacterial zinc metalloregulatory transcriptional repressor, SmtB, are consistent with the idea of vanishingly little or no free zinc in the cell (73). They reveal the surprising finding that SmtB binds Zn(II) with a dissociation equilibrium constant in the

picomolar range. For this site to perform a metalloregulatory switch function *in vivo*, free zinc fluxes would have to occur at the picomolar range or less, *i.e.*, from essentially no free zinc to very small free concentrations of the metal (see Note added in proof). Clearly, a better understanding of how free zinc concentrations change and how this essential metal is sequestered and transported within and between intracellular compartments within the living cell, like that which is currently being pursued in *S. cerevisiae* (41), will obviously have a significant impact on how we design and interpret experiments to further test mechanistic issues of zinc metalloregulation of gene expression.

Note added in proof: Since the time that this paper was accepted for publication, four papers have appeared which directly bear on the subject of this review. One paper implicates reversible phosphorylation of MTF-1 as playing an important role in the regulation of MTF-1 activity (LaRochelle *et al., Biochem J* 353: 591–601, 2001); one summarizes the results of a target gene search for potential MTF-1 regulated genes and the functional significance of the findings (Lichtlen *et al., Nucleic Acids Res* 29: 1514–1523, 2001); another reports that gene expression induced by hypoxia requires MTF-1 and MRE sequences (Green *et al., Cancer Res* 61: 2696–2703, 2001); and one presents new insight on the nuclear–cytoplasmic trafficking of MTF-1 in response to a variety of stress signals, including hydrogen peroxide (Saydam *et al., J Biol Chem* 276: 25487–25495, 2001). The major conclusion from this latter report is that nuclear import of MTF-1 appears necessary but not sufficient for MRE-dependent transcriptional activation. Finally, a recent report documents the femtomolar sensitivity of two zinc metalloregulatory proteins which function together to control zinc homeostasis in *E. coli*; these findings are consistent with the hypothesis that there is essentially no free zinc in these bacteria (Outten and O'Halloran, *Science* 292: 2488–2492, 2001).

ACKNOWLEDGMENTS

Work in the authors' laboratory is supported by grants from the National Institutes of Health (GM42569), the National Science Foundation (DBI-9970232), and the Robert A. Welch Foundation (A-1295).

ABBREVIATIONS

EMSA, electrophoretic mobility shift assay; F1–F6, finger domains 1–6; FRET, fluorescence resonance energy transfer; h-, human; m-, mouse; MRE, metal response element; MT, metallothionein; MTF-1, MRE-binding transcription factor-1; NEM, *N*-ethylmaleimide; ROS, reactive oxygen species; SOD, superoxide dismutase; TFIIIA, transcription factor IIIA.

REFERENCES

1. Andrews GK. Regulation of metallothionein gene expression by oxidative stress and metal ions. *Biochem Pharmacol* 59: 95–104, 2000.
2. Auf der Maur A, Belser T, Elgar G, Georgiev O, and Schaffner W. Characterization of the transcription factor MTF-1 from the Japanese pufferfish (*Fugu ribripes*) reveals evolutionary conservation of the heavy metal response. *Biol Chem* 380: 175–185, 1999.
3. Beard SJ, Hughes MN, and Poole RK. Inhibition of the cytochrome *bd*-terminated NADH oxidase system in *Escherichia coli* K-12 by divalent metal cations. *FEMS Microbiol Lett* 131: 205–210, 1995.
4. Berg JM and Shi Y. The galvanization of biology: a growing appreciation for the roles of zinc. *Science* 271: 1081–1085, 1996.
5. Bittel DC, Dalton T, Samson SLA, Gedamu L, and Andrews GK. 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. *J Biol Chem* 273: 7127–7133, 1998.
6. Bittel DC, Smirnova IV, and Andrews GK. Functional heterogeneity in the zinc fingers of the metalloregulatory transcription factor, MTF-1. *J Biol Chem* 275: 37194–37201, 2000.
7. Brugnera E, Georgiev O, Radtke F, Heuchel R, Baker E, Sutherland GR, and Schaffner W. Cloning, chromosomal mapping and characterization of the human metal-regulatory transcription factor MTF-1. *Nucleic Acids Res* 22: 3167–3173, 1994.
8. Chen X, Agarwal A, and Giedroc DP. Structural and functional heterogeneity among the zinc fingers of human MRE-binding transcription factor-1. *Biochemistry* 37: 11152–11161, 1998.
9. Chen X, Chu M, and Giedroc DP. MRE-binding transcription factor-1 (MTF-1): weak zinc-binding finger domains 5 and 6 modulate the structure, affinity and specificity of the metal-response element (MRE) complex. *Biochemistry* 38: 12915–12925, 1999.

10. Chiang KT, Shinyashiki M, Switzer CH, Valentine JS, Gralla EB, Thiele DJ, and Fukuto JM. Effects of nitric oxide on the copper-responsive transcription factor Ace1 in *Saccharomyces cerevisiae*: cytotoxic and cytoprotective actions of nitric oxide. *Arch Biochem Biophys* 377: 296–303, 2000.
11. Choo Y and Schwabe JWR. All wrapped up. *Nat Struct Biol* 5: 253–255, 1998.
12. Cobine P, Wickramasinghe WA, Harrison MD, Weber T, Solioz M, and Dameron CT. The *Enterococcus hirae* copper chaperone CopZ delivers copper(I) to the CopY repressor. *FEBS Lett* 445: 27–30, 1999.
13. Coleman JE. Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. *Annu Rev Biochem* 61: 897–946, 1992.
14. Culotta VC and Hamer DH. Fine mapping of a mouse metallothionein gene metal response element. *Mol Cell Biol* 9: 1376–1380, 1989.
15. Dalton TP, Li Q, Bittel D, Liang L, and Andrews GK. Oxidative stress activates metal-responsive transcription factor-1 binding activity. Occupancy *in vivo* of metal response elements in the metallothionein-I gene promoter. *J Biol Chem* 271: 25233–25241, 1996.
16. Dalton TP, Bittel D, and Andrews GK. Reversible activation of mouse metal response element-binding transcription factor 1 DNA binding involves zinc interaction with the zinc finger domain. *Mol Cell Biol* 17: 2781–2789, 1997.
17. Dalton TP, Solis WA, Nebert DW, and Carvan MJ. Characterization of the MTF-1 transcription factor from zebrafish and trout cells. *Comp Biochem Physiol [B]* 126: 325–335, 2000.
18. DeFreitas JM, Liba A, Meneghini R, Valentine JS, and Gralla EB. Yeast lacking Cu-Zn superoxide dismutase show altered iron homeostasis. Role of oxidative stress in iron metabolism. *J Biol Chem* 275: 11645–11649, 2000.
19. Eide DJ. The molecular biology of metal ion transport in *Saccharomyces cerevisiae*. *Annu Rev Nutr* 18: 441–469, 1998.
20. Elrod-Erickson M, Rould MA, Nekludova L, and Pabo CO. Zif268–DNA complex refined at 1.6 Å: a model system for understanding zinc finger-DNA interactions. *Structure* 4: 1171–1180, 1996.
21. Fairall L, Schwabe JW, Chapman L, Finch JT, and Rhodes D. The crystal structure of a two-finger peptide reveals an extension to the rules for zinc finger/DNA recognition. *Nature* 366: 483–487, 1993.
22. Foster MP, Wuttke DA, Radhakrishnan L, Case DA, Gottesfeld JM, and Wright PE. Domain packing and dynamics in the DNA complex of the N-terminal zinc fingers of TFIIIA. *Nat Struct Biol* 4: 605–608, 1997.
23. Gaballa A and Helmann JD. Identification of a zinc-specific metalloregulatory protein, Zur, controlling zinc transport operons in *Bacillus subtilis*. *J Bacteriol* 180: 5815–5821, 1998.
24. Ghoshal K, Majumder S, Li Z, Bray TM, and Jacob ST. Transcriptional induction of metallothionein-I and -II genes in the livers of Cu,Zn-superoxide dismutase knockout mice. *Biochem Biophys Res Commun* 264: 735–742, 1999.
25. Ghoshal K, Majumder S, Li Z, Dong X, and Jacob ST. Suppression of Metallothionein gene expression in a rat hepatoma because of promoter-specific DNA methylation. *J Biol Chem* 275: 539–547, 2000.
26. Grotz N, Fox T, Connolly E, Park W, Guerinot ML, and Eide D. Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency. *Proc Natl Acad Sci U S A* 95: 7220–7224, 1998.
27. Günes C, Heuchel R, Georgiev O, Müller K-H, Lichtlen P, Blüthmann H, Marino S, Aguzzi A, and Schaffner W. Embryonic lethality and liver degeneration in mice lacking the metal-responsive transcriptional activator MTF-1. *EMBO J* 17: 2846–2854, 1998.
28. Heuchel R, Radtke F, Georgiev O, Stark G, Aguet M, and Schaffner W. The transcription factor MTF-1 is essential for basal and heavy metal-induced metallothionein gene expression. *EMBO J* 13: 2870–2875, 1994.
29. Houbaviy HB, Usheva A, Shenk T, and Burley SK. Cocrystal structure of YY1 bound to the adeno-associated virus P5 initiator. *Proc Natl Acad Sci U S A* 93: 13577–13582, 1996.
30. Isalan M, Klug A, and Choo Y. Comprehensive DNA recognition through concerted interactions from adjacent zinc fingers. *Biochemistry* 37: 12026–12033, 1998.
31. Kägi JHR and Schäffer A. Biochemistry of metallothionein. *Biochemistry* 27: 8509–8515, 1988.
32. Kim CA and Berg JM. A 2.2 Å resolution crystal structure of a designed zinc finger protein bound to DNA. *Nat Struct Biol* 3: 940–945, 1996.
33. Kobayashi T, Beuchat MH, Lindsay M, Frias S, Palmiter RD, Sakuraba H, Parton RG, and Gruenberg J. Late endosomal membranes rich in lysobisphosphatidic acid regulate cholesterol transport. *Nat Cell Biol* 1: 113–118, 1999.
34. Koizumi S, Suzuki K, Ogra Y, Yamada H, and Otsuka F. Transcriptional activity and regulatory protein binding of metal-responsive elements of the human metallothionein-IIA gene. *Eur J Biochem* 259: 635–642, 1999.
35. Koizumi S, Suzuki K, Ogra Y, Gong P, and Otsuka F. Roles of the zinc fingers and other regions of the transcription factor human MTF-1 in zinc-regulated DNA binding. *J Cell Physiol* 185: 464–472, 2000.
36. Kondo Y, Woo ES, Michalska AE, Choo KH, and Lazo JS. Metallothionein null cells have increased sensitivity to anticancer drugs. *Cancer Res* 55: 2021–2023, 1995.
37. Labbé S, Simard C, and Séguin C. Metallothionein gene regulation in mouse cells. In: *Metal Ions in Gene Regulation*, edited by Silver S and Walden W. New York, NY: Chapman and Hall, 1998, pp. 231–249.
38. Langmade SJ, Ravindra R, Daniels PJ, and Andrews GK. The transcription factor MTF-1 mediates metal-regulation of the mouse ZnT1 gene. *J Biol Chem* 275: 34803–34809, 2000.
39. Lazo JS, Kondo Y, Dellapiazza D, Michalska AE, Choo

KH, and Pitt BR. Enhanced sensitivity to oxidative stress in cultured embryonic cells from transgenic mice deficient in metallothionein I and II genes. *J Biol Chem* 270: 5506–5510, 1995.
40. Lee MS, Gippert GP, Soman KV, Case DA , and Wright PE. Three-dimensional solution structure of a single zinc finger DNA-binding domain. *Science* 245: 635–637, 1989.
41. MacDiarmid CW, Gaither LA, and Eide D. Zinc transporters that regulate vacuolar zinc storage in *Saccharomyces cerevisiae. EMBO J* 19: 2845–2855, 2000.
42. Maret W and Vallee BL. Thiolate ligands in metallothionein confer redox activity on zinc clusters. *Proc Natl Acad Sci U S A* 95: 3478–3482, 1998.
43. Masters BA, Kelly EJ, Quaife CJ, Brinster RL, and Palmiter RD. Targeted disruption of metallothionein I and II genes increases sensitivity to cadmium. *Proc Natl Acad Sci U S A* 91: 584–588, 1994.
44. Morby AP, Turner JS, Huckle JW, and Robinson NJ. SmtB is a metal-dependent repressor of the cyanobacterial metallothionein gene *smtA*: identification of a Zn inhibited DNA-protein complex. *Nucleic Acids Res* 21: 921–925, 1993.
45. Nasir MS, Fahrni CJ, Suhy DA, Kolodsick KJ, Singer CP, and O'Halloran TV. The chemical cell biology of zinc: structure and intracellular fluorescence of a zinc-quinolinesulfonamide complex. *J Biol Inorg Chem* 4: 775–783, 1999.
46. Nekludova L and Pabo CO. Distinctive DNA conformation with enlarged major groove is found in Zn-finger-DNA and protein-DNA complexes. *Proc Natl Acad Sci U S A* 91: 6948–6952, 1994.
47. Nolte RT, Conlin RM, Harrison SC, and Brown RS. Differing roles for zinc fingers in DNA recognition: structure of a six-finger transcription factor IIIA complex. *Proc Natl Acad Sci U S A* 95: 2938–2943, 1998.
48. Ogra Y, Suzuki K, Gong P, Otsuka F, and Koizumi S. Negative regulatory role of Sp1 in metal responsive element-mediated transcriptional activation. *J Biol Chem* 276: 16534–16539, 2001.
49. O'Halloran TV. Transition metals in control of gene expression. *Science* 261: 715–725, 1993.
50. Otsuka F, Okugaito I, Ohsawa M, Iwamatsu A, Suzuki K, and Koizumi S. Novel responses of ZRF, a variant of human MTF-1, to *in vivo* treatment with heavy metals. *Biochim Biophys Acta* 1492: 330–340, 2000.
51. Otvos JD and Armitage IM. Structure of the metal clusters in rabbit liver metallothionein. *Proc Natl Acad Sci U S A* 77: 7094–7098, 1980.
52. Palmiter RD and Findley SD. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. *EMBO J* 14: 639–649, 1995.
53. Palmiter RD, Cole TB, Quaife CJ, and Findley SD. ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular storage. *EMBO J* 15: 1784–1791, 1996.
54. Patzer SI and Hantke K. The ZnuABC high affinity zinc uptake system and its regulator Zur in *Escherichia coli. Mol Microbiol* 28: 1199–1210, 1998.
55. Pavletich NP and Pabo CO. Zinc finger-DNA recognition: crystal structure of a Zif268–DNA complex at 2.1 Å. *Science* 252: 809–817, 1991.
56. Pavletich NP and Pabo CO. Crystal structure of a five-finger GLI-DNA complex: new perspectives on zinc fingers. *Science* 261: 1701–1707, 1993.
57. Pearce LL, Gandley RE, Han W, Wasserloos K, Stitt M, Kanai AJ, McLaughlin MK, Pitt BR, and Levitan ES. Role of metallothionein in nitric oxide signaling as revealed by a green fluorescent fusion protein. *Proc Natl Acad Sci U S A* 97: 477–482, 2000.
58. Pufahl RA, Singer CP, Peariso KL, Lin S-J, Schmidt PJ, Fahrni CJ, Culotta VC, Penner-Hahn JE, and O'Halloran TV. Metal ion chaperone function of the soluble Cu(I) receptor Atx1. *Science* 278: 853–856, 1997.
59. Radtke F, Heuchel R, Georgiev O, Hergersberg M, Gariglio M, Dembic Z, and Schaffner W. Cloned transcription factor MTF-1 activates the mouse metallothionein I promoter. *EMBO J* 12: 1355–1362, 1993.
60. Radtke F, Georgiev O, Müller H-P, Brugnera E, and Schaffner W. Functional domains of the heavy-metal-responsive transcription regulator MTF-1. *Nucleic Acids Res* 23: 2277–2286, 1995.
61. Radtke F, Hug M, Georgiev O, Matsuo K, and Schaffner W. Differential sensitivity of zinc finger transcription factors MTF-1, Sp1 and Kroz-20 to CpG methylation of the their binding sites. *Biol Chem Hoppe Seyler* 377: 47–56, 1996.
62. Rae TD, Schmidt PJ, Pufahl RA, Culotta VC, and O'Halloran TV. Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. *Science* 284: 805–808, 1999.
63. Rae T, Torres AS, Pufahl RA, and O'Halloran TV. Mechanism of Cu,Zn-superoxide dismutase activation by the human metallochaperone CCS. *J Biol Chem* 276: 5166–5176, 2001.
64. Remondelli P and Leone A. Interactions of the zinc-regulated factor (ZiRF-1) with the mouse metallothionein Ia promoter. *Biochem J* 323: 79–85, 1997.
65. Shinyashiki M, Chiang KT, Switzer CH, Gralla EB, Valentine JS, Thiele DJ, and Fukuto JM. The interaction of nitric oxide (NO) with the yeast transcription factor Ace1: a model system for NO-protein thiol interactions with implications to metal metabolism. *Proc Natl Acad Sci U S A* 97: 2491–2496, 2000.
66. Smirnova IV, Bittel DC, Ravindra R, Jiang H, and Andrews GK. Zinc and cadmium can promote rapid nuclear translocation of metal response element-binding transcription factor-1. *J Biol Chem* 275: 9377–9384, 2000.
67. Solioz M and Vulpe C. CPx-type ATPases: a class of P-type ATPases that pump heavy metals. *Trends Biochem Sci* 21: 237–241, 1996.
68. Stuart GW, Searle PF, Chen HY, Brinster RL, and Palmiter RD. A 12–base-pair DNA motif that is repeated several times in metallothionein gene promoters confers metal regulation to a heterologous gene. *Proc Natl Acad Sci U S A* 81: 7318–7322, 1984.
69. Stuart GW, Searle PF, and Palmiter RD. Identification

of multiple metal regulatory elements in mouse metallothionein-I promoter by assaying synthetic sequences. *Nature* 317: 828–831, 1985.
70. Suhy DA, Simon KD, Linzer DIH, and O'Halloran TV. Metallothionein is part of a zinc-scavenging mechanism for cell survival under conditions of extreme zinc deprivation. *J Biol Chem* 274: 9183–9192, 1999.
71. Thelwell C, Robinson NJ, and Turner-Cavet JS. An SmtB-like repressor from *Synechocystis* PCC 6803 regulates a zinc exporter. *Proc Natl Acad Sci U S A* 95: 10728–10733, 1998.
72. Turner JS, Morby AP, Whitton BA, Gupta A, and Robinson NJ. Construction of Zn^{2+}/Cd^{2+} hypersensitive cyanobacterial mutants lacking a functional metallothionein locus. *J Biol Chem* 268: 4494–4498, 1993.
73. VanZile ML, Cosper NJ, Scott RA, and Giedroc DP. The zinc metalloregulatory protein *Synechococcus* PCC7942 SmtB binds a single zinc ion per monomer with high affinity in a tetrahedral coordination geometry. *Biochemistry* 39: 11818–11829, 2000.
74. Westin G and Schaffner W. A zinc-responsive factor interacts with a metal-regulated enhancer element (MRE) of the mouse metallothionein-I gene. *EMBO J* 7: 3763–3770, 1988.
75. Wolfe SA, Neklova L, and Pabo CO. DNA recognition by Cys_2-His_2 zinc finger proteins. *Annu Rev Biophys Biomol Struct* 3: 183–212, 2000.
76. Zhao H and Eide D. The yeast *ZRT1* gene encodes the zinc transporter protein of a high-affinity uptake system induced by zinc limitation. *Proc Natl Acad Sci U S A* 93: 2454–2458, 1997.
77. Zhao H and Eide DJ. Zap1p, a metalloregulatory protein involved in zinc-responsive transcriptional regulation in *Saccharomyces cerevisiae. Mol Cell Biol* 17: 5044–5052, 1997.

Address reprint requests to:
Dr. David P. Giedroc
Center for Advanced Biomolecular Research
2128 TAMU
Department of Biochemistry and Biophysics
Texas A&M University
College Station, TX 77843-2128

Received for publication November 8, 2000; accepted February 9, 2001.

This article has been cited by:

1. Viola Günther, Uschi Lindert, Walter Schaffner. 2012. The taste of heavy metals: Gene regulation by MTF-1. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1823**:9, 1416-1425. [CrossRef]
2. Sharon K. Marr, Katie L. Pennington, Michael T. Marr. 2012. Efficient metal-specific transcription activation by Drosophila MTF-1 requires conserved cysteine residues in the carboxy-terminal domain. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms* . [CrossRef]
3. Mario A. Pennella, David P. GiedrocZinc: DNA-Binding Proteins . [CrossRef]
4. Annie Dubé, Jean-François Harrisson, Geneviève Saint-Gelais, Carl Séguin. 2011. Hypoxia acts through multiple signaling pathways to induce metallothionein transactivation by the metal-responsive transcription factor-1 (MTF-1). *Biochemistry and Cell Biology* **89**:6, 562-577. [CrossRef]
5. Rajani Salunke, Nidhi Rawat, Vijay Kumar Tiwari, Kumari Neelam, Gursharn Singh Randhawa, Harcharan Singh Dhaliwal, Partha Roy. 2011. Determination of bioavailable-zinc from biofortified wheat using a coupled in-vitro digestion/Caco-2 reporter-gene based assay. *Journal of Food Composition and Analysis* . [CrossRef]
6. Meng-Chieh Lin, Ya-Chuan Liu, Ming F. Tam, Yu-Ju Lu, Ya-Ting Hsieh, Lih-Yuan Lin. 2011. PTEN interacts with metal-responsive transcription factor 1 and stimulates its transcriptional activity. *Biochemical Journal* . [CrossRef]
7. Lilit Atanesyan, Viola Günther, Susan E. Celniker, Oleg Georgiev, Walter Schaffner. 2011. Characterization of MtnE, the fifth metallothionein member in Drosophila. *JBIC Journal of Biological Inorganic Chemistry* . [CrossRef]
8. Qiang Ma. 2010. Transcriptional responses to oxidative stress: Pathological and toxicological implications. *Pharmacology & Therapeutics* **125**:3, 376-393. [CrossRef]
9. Sara Balesaria, Bala Ramesh, Harry McArdle, Henry K. Bayele, Surjit K.S. Srai. 2010. Divalent metal-dependent regulation of hepcidin expression by MTF-1. *FEBS Letters* **584**:4, 719-725. [CrossRef]
10. Yinzhi Zhao, Lijun Chen, Song Gao, Paul Toselli, Phillip Stone, Wande Li. 2010. The critical role of the cellular thiol homeostasis in cadmium perturbation of the lung extracellular matrix. *Toxicology* **267**:1-3, 60-69. [CrossRef]
11. Tehila Kaisman-Elbaz, Israel Sekler, Daniel Fishman, Nataly Karol, Michal Forberg, Nicole Kahn, Michal Hershfinkel, William F. Silverman. 2009. Cell death induced by zinc and cadmium is mediated by clusterin in cultured mouse seminiferous tubules. *Journal of Cellular Physiology* **220**:1, 222-229. [CrossRef]
12. Dhananjay Shukla , Saurabh Saxena , Purushotman Jayamurthy , Mustoori Sairam , Mrinalini Singh , Swatantra Kumar Jain , Anju Bansal , Govindaswamy Ilavazaghan . 2009. Hypoxic Preconditioning with Cobalt Attenuates Hypobaric Hypoxia-Induced Oxidative Damage in Rat Lungs. *High Altitude Medicine Biology* **10**:1, 57-69. [Abstract] [Full Text PDF] [Full Text PDF with Links]
13. Kuppusamy Balamurugan, Haiqing Hua, Oleg Georgiev, Walter Schaffner. 2009. Mercury and cadmium trigger expression of the copper importer Ctr1B, which enables Drosophila to thrive on heavy metal-loaded food. *Biological Chemistry* **390**:2, 109-113. [CrossRef]
14. G WAN, W CHEUK, K CHAN. 2009. Differential regulation of zebrafish metallothionein-II (zMT-II) gene transcription in ZFL and SJD cell lines by metal ions#. *Aquatic Toxicology* **91**:1, 33-43. [CrossRef]
15. W. Liu, Y.S. Yang, D. Francis, H.J. Rogers, P. Li, Q. Zhang. 2008. Cadmium stress alters gene expression of DNA mismatch repair related genes in Arabidopsis seedlings. *Chemosphere* **73**:7, 1138-1144. [CrossRef]
16. Uschi Lindert, Lucas Leuzinger, Kurt Steiner, Oleg Georgiev, Walter Schaffner. 2008. Characterization of Metal-Responsive Transcription Factor (MTF-1) from the Giant Rodent Capybara Reveals Features in Common with Human as Well as with Small Rodents (Mouse, Rat). Short Communication. *Chemistry & Biodiversity* **5**:8, 1485-1494. [CrossRef]

17. Bo-Yeon Park, Jayong Chung. 2008. Effects of various metal ions on the gene expression of iron exporter ferroportin-1 in J774 macrophages. *Nutrition Research and Practice* **2**:4, 317. [CrossRef]
18. Julia M. Morahan, Bing Yu, Ronald J Trent, Roger Pamphlett. 2007. Genetic susceptibility to environmental toxicants in ALS. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* **144B**:7, 885-890. [CrossRef]
19. Irina Schmidt, Michael Fähling, Benno Nafz, Angela Skalweit, Bernd-Joachim Thiele. 2007. Induction of translationally controlled tumor protein (TCTP) by transcriptional and post-transcriptional mechanisms. *FEBS Journal* **274**:20, 5416-5424. [CrossRef]
20. John H. Laity, Glen K. Andrews. 2007. Understanding the mechanisms of zinc-sensing by metal-response element binding transcription factor-1 (MTF-1). *Archives of Biochemistry and Biophysics* **463**:2, 201-210. [CrossRef]
21. Macus Tien Kuo, Helen H. W. Chen, Im-Sook Song, Niramol Savaraj, Toshihisa Ishikawa. 2007. The roles of copper transporters in cisplatin resistance. *Cancer and Metastasis Reviews* **26**:1, 71-83. [CrossRef]
22. Matthew B. Wolf, John W. Baynes. 2007. Cadmium and mercury cause an oxidative stress-induced endothelial dysfunction. *BioMetals* **20**:1, 73-81. [CrossRef]
23. Kyong-Son MIN. 2007. The Physiological Significance of Metallothionein in Oxidative Stress. *YAKUGAKU ZASSHI* **127**:4, 695-702. [CrossRef]
24. G. Bertin, D. Averbeck. 2006. Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). *Biochimie* **88**:11, 1549-1559. [CrossRef]
25. Wolfgang Maret . 2006. Zinc Coordination Environments in Proteins as Redox Sensors and Signal Transducers. *Antioxidants & Redox Signaling* **8**:9-10, 1419-1441. [Abstract] [Full Text PDF] [Full Text PDF with Links]
26. Mario A. Pennella, David P. GiedrocZinc: DNA-Binding Proteins . [CrossRef]
27. M STITT, K WASSERLOOS, X TANG, X LIU, B PITT, C STCROIX. 2006. Nitric oxide-induced nuclear translocation of the metal responsive transcription factor, MTF-1 is mediated by zinc release from metallothionein. *Vascular Pharmacology* **44**:3, 149-155. [CrossRef]
28. K MIN, F MORISHITA, N TETSUCHIKAWAHARA, S ONOSAKA. 2005. Induction of hepatic and renal metallothionein synthesis by ferric nitrilotriacetate in mice: the role of MT as an antioxidant. *Toxicology and Applied Pharmacology* **204**:1, 9-17. [CrossRef]
29. Kazuo Kobayashi, Rumi Shida, Tatsuya Hasegawa, Masahiko Satoh, Yoshiyuki Seko, Chiharu Tohyama, Junji Kuroda, Nobuo Shibata, Nobumasa Imura, Seiichiro Himeno. 2005. Induction of Hepatic Metallothionein by Trivalent Cerium: Role of Interleukin 6. *Biological & Pharmaceutical Bulletin* **28**:10, 1859-1863. [CrossRef]
30. K. Balamurugan, D. Egli, A. Selvaraj, B. Zhang, O. Georgiev, W. Schaffner. 2004. Metal-responsive transcription factor (MTF-1) and heavy metal stress response in Drosophila and mammalian cells: a functional comparison. *Biological Chemistry* **385**:7, 597-603. [CrossRef]
31. Y. Wang, I. Lorenzi, O. Georgiev, W. Schaffner. 2004. Metal-responsive transcription factor-1 (MTF-1) selects different types of metal response elements at low vs. high zinc concentration. *Biological Chemistry* **385**:7, 623-632. [CrossRef]
32. 2003. Trend of Most Cited Papers (2001-2002) in ARS. *Antioxidants & Redox Signaling* **5**:6, 813-815. [Citation] [Full Text PDF] [Full Text PDF with Links]
33. Masaru Hasumi, Kazuhiro Suzuki, Hiroshi Matsui, Hidekazu Koike, Kazuto Ito, Hidetoshi Yamanaka. 2003. Regulation of metallothionein and zinc transporter expression in human prostate cancer cells and tissues. *Cancer Letters* **200**:2, 187-195. [CrossRef]