The Transcription Factor MTF-1 Mediates Metal Regulation of the Mouse ZnT1 Gene*

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Zinc metabolism is controlled by uptake and efflux, as well as by storage in peripheral tissues, but the mechanisms regulating homeostasis of this metal are poorly defined. Zinc absorption occurs in the intestinal mucosa (1), and zinc is primarily lost in the bile-pancreatic secretions (2, 3). Four mammalian genes involved in zinc transport have been identified (4). Zinc transporters (ZnT)1 1–4 are proteins with six membrane-spanning domains; these four proteins function in the efflux or vesicular storage of zinc (5, 6). Mouse ZnT2 causes the vesicular accumulation of zinc in endosomal vesicles (5) and is most similar in structure to ZnT3, which is responsible for the accumulation of zinc in synaptic vesicles in the brain (7, 8). Targeted deletion of ZnT3 is not lethal (8). ZnT4 was identified during a search for the Lethal Milk locus in the mouse (9). This zinc effluxer is highly expressed in the mammary gland, but may be involved in more general zinc homeostasis in the adult (9). ZnT1 functions to efflux zinc from cells, is localized to the plasma membrane, and is expressed ubiquitously (5, 10). ZnT1 is an essential gene, and homozygous knockout of the ZnT1 gene is lethal to the embryo.2 Zinc induction of ZnT1 mRNA had been documented in cultured neurons (11), and in the rat intestine after oral gavage with zinc (12, 13). Furthermore, ZnT1 expression in enterocytes can be regulated by dietary zinc (12). These preliminary studies suggested that zinc may regulate ZnT1 gene expression.

In higher eukaryotes, the best understood metal-regulated genes are the metallothioneins (MT) (for review, see Ref. 14). Transcription of the mouse MT-I gene, for example, is regulated by zinc and cadmium, and this regulation is mediated by metal response element-binding transcription factor-1 (MTF-1) (15). MTF-1 is a six zinc-finger (Cys2His2) transcription factor, which functions as a sensor of intracellular zinc (for review, see Ref. 14). MTF-1 is activated by zinc to bind to metal response elements (MREs) in the MT-I promoter, resulting in an increased rate of transcription of this gene (15–17). Cadmium activation of MT-I gene expression also requires MTF-1. In the present study, the hypothesis that zinc and cadmium regulate ZnT1 gene expression was tested and the potential role of MTF-1 in this response was examined. The ZnT1 gene was found to be responsive to zinc excess and deficiency, as well as to cadmium. These metals rapidly induced the coordinated synthesis of ZnT1 and MT-I mRNAs in cultured cells. In vitro DNA-binding assays demonstrated that recombinant mouse MTF-1 can bind to the MRE sequences present in the mouse ZnT1 promoter and studies of MTF-1 knockout mice and mouse embryonic fibroblast cells revealed an essential role for MTF-1 in metal responsiveness of these genes.

EXPERIMENTAL PROCEDURES

Materials—Bovine serum albumin (BSA) and actinomycin D were purchased from Sigma. Dulbecco’s modified Eagle’s medium (DMEM)

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1 The abbreviations used are: ZnT, zinc transporter; BSA, bovine serum albumin; DMEM, Dulbecco’s modified Eagle’s medium; EMSA, electromophoretic mobility shift assay; FBS, fetal bovine serum; MEF, mouse embryonic fibroblast; MRE, metal response element; MT, metallothionein; MTF-1, metal response element-binding transcription factor-1; ZnA, zinc-adequate diet; ZnD, zinc-deficient diet; bp, base pair(s); RT, reverse transcriptase; PCR, polymerase chain reaction; d, day.

2 R. D. Palmer, personal communication.
were preincubated in DMEM containing 1% BSA and 10 effects of protein and RNA synthesis inhibitors on metal induction, cells BSA plus 100 bated overnight in DMEM containing 2% FBS, 2% Chelex-treated FBS, 70% confluent. Cells were washed twice with DMEM and then incu-
diets each contain about 18 deficient (ZnD), 1 ppm zinc; zinc-adequate (ZnA), 50 ppm zinc. These
detail previously (19). Zinc levels in the diets were as follows: zinc-
were approved by our Institutional Animal Care and Use Committee. In
the care and use of experimental animals, and all animal experiments
ducted in accordance with National Institutes of Health guidelines for
(20). On d12 and d14 mice were sacrificed by cervical dislocation and
embryo, DNA was extracted and analyzed by PCR as described in detail
analysis.

Embryos and visceral yolk sacs were harvested on d12. For genotyping each


was used to verify the ZnT1 cDNA clone.

for cDNA synthesis, and the primers used had the following sequence:

TGACAATCTGGAAGCGGAAGACAAC (sense), GGAAGCGGGGTC-

MTF-1 Modulates ZnT1 Gene Expression

RNA Isolation, Northern Blot, and Quantitative Real-time RT-PCR

was purchased from BioWhittaker, Inc. (Walkersville, MD). Fetal bo-
vine serum (FBS) was purchased from Atlanta Biologicals (Norcross, GA). Chelex-100 chelating resin was purchased from Bio-Rad. Radio-active cytidine triphosphate (α[32P]CTP, 600 Ci/mmol) was purchased from PerkinElmer Life Sciences. Cycloheximide was purchased from Bio-Pan wurde purchased from BioWhittaker, Inc. (Walkersville, MD). Mouse embryo fibroblasts (MEF), derived from
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analyses—Total RNA from cell pellets and tissues was isolated using an RNeasy Maxi kit according to the manufacturer’s instructions (Qiagen, Valencia, CA). Total RNA from yolk sacs (4 per group) was isolated using the RNeasy Mini kit according to the manufacturer’s instructions (Qiagen). To remove residual DNA, total RNA samples were precipi-
tated with an equal volume of 2 M ammonium acetate (pH 5.2) (23) and chilled previously (24), before a final ethanol precipitation. Poly(A)+ RNA was enriched from 100–250 µg of total RNA using the Oligotex mRNA mini kit (Qiagen). No attempt was made to quantify the amount of poly(A)+ RNA obtained from each sample, but a β-actin probe was used as a control to normalize for RNA amount, integrity, and transfer efficiency (19). Hybrids were detected by autoradiography at −70 °C with intensifying screens and quantitated by radioimage analysis (Molecular Dynamics, Sunnyvale, CA). In each experiment, a duplicate gel was stained with acridine orange to verify integrity and equal loading of RNA and blots were co-hybridized with MT-1 and β-actin probes as internal controls.

PCR products were covalently crosslinked with UV light to prevent DNA degradation and detected by ethidium bromide staining. PCR products were visualized on 1% agarose gels and quantitated using a Bio-Rad Gel Documentation System (Bio-Rad Laboratories, Hercules, CA). RT-PCR products were quantitated by scanning the gels and calculating the fraction of the total band intensity corresponding to each fragment.

Electrophoretic Mobility Shift Assay (EMSA) of MTF-1 Binding to ZnT1 MREs—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 described previ-
ously (28) and Sp6 RNA polymerase according to the manufacturer’s suggestions (29). EMSA was performed as described in detail (16, 28, 29). MTF-1 in vitro transcription/translation reaction (1 µl of a 50-µl reaction) was incubated in buffer containing 12 mM HEPES (pH 7.9), 60 mM KCl, 0.5 mM dithiothreitol, 12% glycerol, 5 mM MgCl2, 4 µg of dI-dC, 800 Ci/mmol) was purchased from DuPont NEN (Boston, MA). All other reagents were purchased from Sigma.

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of increasing molar excess of unlabeled MRE competitor (30). The amount of radioactivity in the specific MRE-binding complex was quantitated by radioimaging the dried gel, and the approximate molar excess of competitor required to achieve 50% inhibition was calculated.

The MRE oligonucleotide sequences used were as follows: GATCCAGGGAGCTCTGGACACGCGGACAAGATGA, MRE-a (31); GATCCAGGGAGCTCTGGACACGCGGACAAGATGA, mutMRE (28); GATCCAGGGAGCTCTGGACACGCGGACAAGATGA, MRE-a (5); ATTCACACTGCCGGGAAAACGCT, MRE-b (5).

The bold bases are the conserved core bases in functional MREs, and underlined bases deviate from the consensus MRE core sequence (TGCRCNC).

RESULTS
Zinc and Cadmium Coordinate Induce ZnT1 and MT-I Gene Expression in Cultured Cells—The effects of zinc and cadmium on ZnT1 mRNA levels in mouse Hepa cells and MEFs were examined by Northern blotting and real-time RT-PCR (Figs. 1 and 2). Two approaches to this problem were taken in the experiment shown in Fig. 1. The effects of removing metal ions from the culture medium (2% Chelex-treated FBS), as well as the effects of exposure to increased concentrations of the metals zinc and cadmium, were examined. FBS normally contains about 38 \( \mu \text{M} \) zinc (32); therefore DMEM plus 2% FBS is expected to contain about 0.8 \( \mu \text{M} \) zinc. Chelex treatment removes this zinc, as well as other metal ions (iron and copper) from the FBS. DMEM replenishes essential divalent cations and iron, but not copper or zinc.

Northern blotting of poly(A)\(^+\) RNA detected two ZnT1 transcripts (5 and 2 kilobase pairs), as reported previously (10, 12). Radioimage analysis of membranes suggested that the abundance of these transcripts is coordinately regulated. In this study, the data presented were obtained for the 2-kilobase pair ZnT1 transcript. Overnight incubation of Hepa cells in medium containing 2% Chelex-treated FBS led to a 2.5-fold reduction in the steady state levels of ZnT1 mRNA (Fig. 1). MT-I mRNA was similarly reduced. In contrast, exposure of Hepa cells to excess zinc (100 \( \mu \text{M} \) ZnSO\(_4\)) or to cadmium (10 \( \mu \text{M} \) CdCl\(_2\)) resulted in the rapid (3 h) and dramatic induction of ZnT1 mRNA. These concentrations of zinc and cadmium result in maximal induction of MT-I mRNA in Hepa cells (23). The magnitude of induction of ZnT1 mRNA was increased in cells cultured in medium containing Chelex-treated FBS. Zinc or cadmium each caused a 12-fold induction in control cultures and a 26- or 31-fold induction, respectively, in “metal-deficient” cultures.

MEFs were also examined for the effects of zinc and cadmium on ZnT1 gene expression (Fig. 2). In these experiments, Hepa cells and MEFs were incubated overnight in DMEM containing 1% BSA before treatment with metals. The zinc concentration in 1% BSA is about 0.15 \( \mu \text{M} \), but little or no iron or copper are present (data from Sigma). Iron is provided by the DMEM. Analysis of the time course for induction of ZnT1 mRNA revealed that peak mRNA levels were detected at 3 h after addition of either 100 \( \mu \text{M} \) ZnSO\(_4\) or 10 \( \mu \text{M} \) CdCl\(_2\) (Fig. 2). This was noted in mouse Hepa cells, as well as in MEFs (Fig. 2, panels A and B and panel C, respectively).

Real-time RT-PCR was also used to quantitate the effects of these metals on the relative abundance of ZnT1 and \( \beta \)-actin mRNA in the above RNA samples (Fig. 2D). Because Northern blotting experiments required isolation of poly(A)\(^+\) RNA in order to detect ZnT1 mRNA in control cells, it was important to test the possibility that metal ions may cause the rapid polyadenylation of preexisting ZnT1 mRNA in these cells. However, the results obtained using real-time RT-PCR of total RNA from control and metal-treated cells agreed with the Northern blotting results shown above.

In these experiments, zinc and cadmium coordinately induced MT-I and ZnT1 mRNAs. Analysis of dose-response curves for these metals revealed that as little as 3 \( \mu \text{M} \) cadmium and 60 \( \mu \text{M} \) zinc were minimal effective concentrations for the induction of both of these mRNAs (data not shown). To further examine the mechanisms of this induction, the effects of RNA and protein synthesis inhibitors were examined (Fig. 3). Inhi-
MTF-1 Modulates ZnT1 Gene Expression

The ZnT1 Gene Is Actively Expressed in the Visceral Yolk Sac and Placenta of the Developing Mouse Embryo—Nothing is known about the normal patterns of expression of the ZnT1 gene during development of the embryo. However, the mouse MT genes are subjected to regulation in a cell-specific manner in the developing conceptus and in the adult (22, 36–38). Heightened expression of the MT-I gene occurs first in the endodermal cells of the visceral yolk sac of the mouse embryo (36), and

**MTF-1 Is Essential for Heavy Metal Induction of ZnT1 Gene Expression in Cultured Cells—**MEFs derived from wild-type embryos, and from embryos homozygous for targeted disruption of the MT-I gene were examined (Fig. 5) to test the hypothesis that MTF-1 regulates metal induction of ZnT1 gene expression. As in Fig. 2, cells were incubated overnight in DMEM containing 1% BSA before treatment with 100 μM ZnSO₄ or 10 μM CdCl₂. Northern blotting revealed that the steady state levels of ZnT1 and MT-I mRNAs were significantly reduced in untreated MTF-1 knockout MEFs compared with wild-type MEFs. Furthermore, neither zinc (Fig. 5A) nor cadmium (Fig. 5B) caused an increase in the levels of these mRNAs in the MTF-1 knockout cells. In contrast, these metals rapidly (3 h) induced both ZnT1 and MT-I mRNAs in the wild-type MEFs. These results establish that MTF-1 mediates both basal expression and metal induction of both ZnT1 and MT-I genes in MEF cell lines.

**The Binding of MTF-1 to MREs from the ZnT1 Promoter**—The above experiments suggest that zinc and cadmium may activate ZnT1 and MT-I gene transcription by a common mechanism. Both the MT-I (35) and ZnT1 (10) promoters contain MRE consensus sequences, which represent potential binding sites for the transcription factor MTF-1 (31). Two MRE consensus sequences are located at −87 bp (MRE-a) and −116 bp (MRE-b) relative to the transcription start point in the ZnT1 promoter. EMSA was used to determine whether recombinant mouse MTF-1 can bind to these MREs (Fig. 4), and to compare that binding with its binding to MRE-s, a consensus MRE that represents a high affinity MTF-1 binding site (31); MRE-a, sequence at −87 bp relative to the transcription start site in the mouse ZnT1 promoter; MRE-b, sequence at −116 bp in the mouse ZnT1 promoter; mutMRE, mutant MRE-s that does not bind MTF-1. After 15 min at 4°C, the reactions were subjected to PAGE. The amount of MTF-1-MRE complex was quantitated by phosphorimage analysis. Competition EMSA in which labeled MRE-a or MRE-b was incubated with MTF-1 in the presence of an increasing molar excess of unlabeled competitor (MRE-s). Competition results were compared with the ability of unlabeled MRE-s to compete with itself for MTF-1 binding. The amount of MTF-1-MRE complex was quantitated by phosphorimage analysis (data not shown).

The binding of MTF-1 to MRE-a and MRE-b from the ZnT1 promoter was further examined by competition EMSA, in which labeled MRE-a, MRE-b, or MRE-s was incubated with zinc-activated MTF-1 in the presence of an increasing molar excess of unlabeled competitor (MRE-s). The results demonstrated that MTF-1 binds with similar avidity to MRE-a, MRE-a, and MRE-b (data not shown).

**MTF-1 Can Bind Avidly to MREs from the Mouse ZnT1 Promoter**—The above experiments suggest that zinc and cadmium may activate ZnT1 and MT-I gene transcription by a common mechanism. Both the MT-I (35) and ZnT1 (10) promoters contain MRE consensus sequences, which represent potential binding sites for the transcription factor MTF-1 (31). Two MRE consensus sequences are located at −87 bp (MRE-a) and −116 bp (MRE-b) relative to the transcription start point in the ZnT1 promoter. EMSA was used to determine whether recombinant mouse MTF-1 can bind to these MREs (Fig. 4), and to compare that binding with its binding to MRE-s, a consensus MRE that represents a high affinity MTF-1 binding site (31).

Recombinant mouse MTF-1 bound to MRE-s, MRE-a, and MRE-b in a zinc-dependent manner (Fig. 4). Under these conditions MTF-1 binding was dependent on an intact core sequence TGCRCNC (35) and did not occur with a mutant MRE in which the first three bases are mutated (28). However, the MRE-a core sequence TGCRCNC differs from the consensus at the fifth base. Cross-competition experiments demonstrated that mutant MRE could not compete for binding to MRE-a, MRE-b, or MRE-s, whereas a 160-fold molar excess of MRE-s eliminated binding.

**Fig. 3.** The effects of actinomycin D and cycloheximide on the metal induction of ZnT1 and MT-I mRNAs in mouse Hepa and MEF cells. Mouse Hepa and MEF cells were preincubated for 40 min in DMEM containing 1% BSA and actinomycin D (Act D; 10 μg/ml) or cycloheximide (Chx; 10 μg/ml) before the addition of metals to the culture medium. The cells were treated with either 100 μM zinc or 10 μM cadmium for 3 h before isolation of total RNA. Total RNA was subjected to formaldehyde-agarose gel electrophoresis, blotted onto a nylon membrane, and hybridized with 32P-labeled ZnT1, MT-I, and β-actin cRNA probes. Northern blotting was performed using total RNA, which precluded detection of ZnT1 transcripts in control cells.

**Fig. 4.** EMSA detection of mouse MTF-1 binding to MRE sequences from the ZnT1 promoter. Recombinant mouse MTF-1 was synthesized in vitro in a TnT lysate, as described (28). The DNA binding activity of MTF-1 was activated with zinc (+), and binding reactions were assembled with contained the following labeled double-stranded oligonucleotides: MRE-s, a consensus MRE oligonucleotide that has a specific, high affinity MTF-1 binding site (31); MRE-a, sequence at −87 bp relative to the transcription start site in the mouse ZnT1 promoter; MRE-b, sequence at −116 bp in the mouse ZnT1 promoter; mutMRE, mutant MRE-s that does not bind MTF-1. After 15 min at 4°C, the reactions were subjected to PAGE. The amount of MTF-1-MRE complex was quantitated by phosphorimage analysis. Competition EMSA in which labeled MRE-a or MRE-b was incubated with MTF-1 in the presence of an increasing molar excess of unlabeled competitor (MRE-s). Competition results were compared with the ability of unlabeled MRE-s to compete with itself for MTF-1 binding. The amount of MTF-1-MRE complex was quantitated by phosphorimage analysis (data not shown).
MTF-1 is essential for this expression. MT-I mRNA is also particularly abundant in the placenta, but MTF-1 is not essential for that expression.

Northern blot analysis of ZnT1 mRNA in various tissues of the conceptus revealed that this mRNA is ubiquitously expressed, but is particularly abundant in the d14 visceral yolk sac and placenta (Fig. 6). This mRNA is 8–11-fold more abundant in visceral yolk sac and placenta, respectively, than in the embryo. By comparison, ZnT1 mRNA is very rare in the cell lines examined, which necessitated the use of poly(A) RNA for Northern blotting.

The ZnT1 Gene Is Regulated, in Part, by Dietary Zinc and MTF-1 in the Visceral Yolk Sac of the Developing Mouse Embryo—The effects of maternal dietary zinc deficiency during pregnancy on ZnT1 gene expression were examined (Fig. 7). Pregnant females were fed a normal ZnA (50 ppm zinc) or a ZnD diet (1 ppm zinc) beginning on d8 of pregnancy. The ZnD diet causes about 20% of the embryos to develop abnormally under these conditions (20). RNA was extracted from visceral yolk sacs and placentae collected at d12 and d14 of pregnancy. Northern blot analysis (Fig. 7) revealed that ZnT1 mRNA levels in visceral yolk sac were reduced about 4.3-fold by d14 in the mice fed the ZnD diet. In contrast, MT-I mRNA levels were reduced 22-fold by d14. Dietary zinc deficiency had little effect on ZnT1 mRNA levels (1.5–2-fold reduction) in the d14 placentas (data not shown).

The role of MTF-1 in regulating ZnT1 gene expression in the visceral endoderm was examined using mice with targeted mutations in the MTF-1 gene (18). Although homozygous MTF-1 (−/−) knockout embryos die at d14 (18), they develop normally up until that time (39). Heterozygous MTF-1 knockout (+/−) mice were inbred and the embryos were examined at d12 of pregnancy. The genotype of each embryo was determined by PCR and RNA was extracted from visceral yolk sacs from embryos with the same genotype (Fig. 8). Northern blotting revealed that ZnT1 mRNA levels were reduced 4–6-fold in visceral yolks from homozygous embryos. In contrast, MT-I mRNA was essentially undetectable in these same RNA samples, as reported previously. Thus, MTF-1 and dietary zinc regulate, in part, ZnT1 gene expression in the visceral yolk sac. In contrast, this transcription factor is essential for expression of the MT-I gene in this tissue.

DISCUSSION

These studies demonstrate that expression of the mouse ZnT1 gene is regulated, in part, by the heavy metals zinc and cadmium, and suggest that MTF-1 is the transcription factor that mediates this response. Thus, MTF-1 coordinates the expression of ZnT1 and MT-I genes in the visceral yolk sac.
genotype of each embryo was determined by PCR. Inbred and embryos and yolk sacs were harvested on d12. The MTF-1 knockout embryos. Visceral yolk sac from wild-type, heterozygous, and homozygous homozygous for MTF-1 knockout alleles. RNA was isolated from the MTF-1 alleles; 1/2, heterozygous for MTF-1 knockout allele; -/-, homozygous for MTF-1 knockout alleles. RNA was isolated from the yolk sac (4 each) from each respective genotype. Total RNA (3 µg) was subjected to formaldehyde-agarose gel electrophoresis, blotted onto a nylon membrane, and hybridized with 32P-labeled ZnT1, MT-I, and β-actin cRNA probes. Hybrids were detected by autoradiography and quantitated by radiomage analysis.

pression of genes that play roles in zinc homeostasis, as well as in protection from metal toxicity. Exposure of cells to excess zinc results in the increased expression of MT genes, which encode the major intracellular zinc storage proteins (40), and the expression of ZnT1, which effluxes the metal from the cell (10). Reciprocally, under conditions of zinc deprivation, MTs are degraded to provide a biologically active labile pool of zinc (19, 20), and the efflux of zinc via ZnT1 is attenuated (4, 10) leading to conservation of this metal in the cell. However, unlike MT-I and -II (41), MTF-1 (18) and ZnT12 are essential for embryonic development of the mouse. This suggests that metal efflux plays a more important role during development of the embryo than does metal storage. Remarkably, cadmium also coordinately regulates the expression of MT-I and ZnT1 genes, suggesting that ZnT1 may also play a role in protecting from cadmium toxicity, as does MT (41, 42). Consistent with this concept are the findings that overexpression of ZnT1 protects cells from zinc toxicity (10), and that zinc-resistant Hepa cells overexpress MT as well as ZnT1. Whether these cells also display increased efflux of cadmium and increased resistance to cadmium toxicity remains to be determined. A recent study of the ZnTA gene in *Escherichia coli*, which is a cadmium/zinc-exporting P1-type ATPase, is also regulated by zinc and cadmium (43).

Whether MTF-1 directly or indirectly regulates ZnT1 gene expression remains to be determined, and the data presented herein cannot formally exclude either possibility. However, several lines of evidence are consistent with the concept that MTF-1 directly regulates ZnT1 gene expression in response to metals. First, both zinc and cadmium induce the rapid and coordinated synthesis of ZnT1 and MT-I mRNAs in cultured cells that contain MTF-1, but not in those lacking MTF-1. Second, both ZnT1 and MT-I mRNAs are specifically elevated in the visceral endoderm during early development of the embryo, both genes respond to dietary zinc deficiency, and both are reduced in mice lacking MTF-1. Third, MTF-1 can bind with avidity to two MREs found in the ZnT1 promoter, as it can with MRE sequences from the mouse MT-I promoter. Despite these findings, previous transfection studies using the ZnT1 promoter did not demonstrate metal regulation (10). The reason for this discrepancy warrants further investigation. Clearly, there are similarities and also distinct differences in the mechanisms of regulation of the ZnT1 and MT-I genes. Unlike the mouse MT-I promoter, which contains five functional MREs in the proximal 200-bp promoter, the ZnT1 proximal promoter contains only two MRE sequences. MTF-1 plays an important, but nonessential, role in regulating the ZnT1 gene in visceral endoderm cells in *vivo*. Thus, the basal level of expression of the ZnT1 gene is clearly dependent on transcription factors other than MTF-1. One potential binding site for the zinc-finger transcription factor Sp1 is present upstream of the MREs in the proximal MT-I promoter, whereas at least four such sites are found in the ZnT1 promoter. Further studies of the structure and function of the ZnT1 promoter are required.

The finding that the visceral yolk sac actively expresses both the ZnT1 gene and the MT-I/II genes suggests that this organ plays an important role in zinc homeostasis, and protection from excess zinc during pregnancy. Preliminary immunolocalization studies using rat ZnT1 antisera (provided by R. J. Cousins, University of Florida, Gainsville, FL) detected immunoreactivity specifically in the visceral endoderm layer of the yolk sac. These cells are also the site of synthesis of MT (36). Visceral endoderm cells are the second cell type to differentiate from the primitive endoderm of the inner cell mass and they form the secretory layer of the visceral yolk sac, which surrounds the embryo until late in pregnancy (d19). These cells are responsible for the synthesis of serum proteins, and the visceral yolk sac is the first site of hemopoiesis. The visceral endoderm plays a nutritive and supportive role for embryonic development of the mouse. Previous studies demonstrated that the mouse MT genes become responsive to metal ions first at the morula/blastocyst stage of development. Given the role of MTF-1 in metal regulation of MT as well as ZnT1 genes, these studies suggest that ZnT1 gene expression may also be activated and responsive to metals first at this stage of preimplantation development. Further studies are required to address this possibility.

In summary, these studies demonstrate that the mouse ZnT1 gene can be regulated by zinc as well as cadmium, and that this regulation is dependent on the transcription factor MTF-1. It was further demonstrated that expression of the ZnT1 gene is highly active in the visceral yolk sac of the developing embryo, and this expression is partially dependent on MTF-1 and dietary zinc. MTF-1 was known to regulate expression of the MT-I/II genes in mice, but the MT genes are nonessential. In contrast, the MTF-1 gene is essential for development, which suggested that this transcription factor also regulates the expression of an essential gene(s). One such gene is the ZnT1 gene.

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