Cadmium-mediated Activation of the Metal Response Element in Human Neuroblastoma Cells Lacking Functional Metal Response Element-binding Transcription Factor-1*

Waihei A. Chu‡, Jeffrey D. Moehlenkamp‡§, Doug Bittle†¶, Glen K. Andrews†, and Jeffrey A. Johnson‡**

From the ‡Departments of Pharmacology, Toxicology, and Therapeutics and the §Departments of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas 66160-7417

Metal response element-binding transcription factor-1 (MTF-1) binds specifically to metal response elements (MREs) and transactivates metallothionein (MT) gene expression in response to zinc and cadmium. This investigation contrasts the mechanism of mouse MT gene (mMT-I) promoter activation by cadmium and zinc in IMR-32 human neuroblastoma cells to determine whether MTF-1 binding to the MRE is necessary for activation by these metals. Cadmium activated a mMT-1 promoter (−150 base pairs) luciferase reporter 20–25-fold through a MRE-dependent mechanism. In contrast, zinc had little effect on the mMT-1 luciferase reporter. IMR-32 cells lacked MRE binding activity, and treatment with zinc in vitro or in vivo did not generate a MTF-1-MRE complex, suggesting that IMR-32 cells lack functional MTF-1. Overexpression of mMTF-1 generated a zinc-mediated induction of the MRE without affecting cadmium activation. Because no other transition metals tested activated the MRE, this effect appeared to be cadmium-specific. These data demonstrate that in IMR-32 human neuroblastoma cells, zinc and cadmium can use independent mechanisms for activation of the mMT-I promoter and cadmium-mediated MRE activation is independent of MTF-1 and zinc.

Metallothioneins (MTs) constituting a conserved family of cysteine-rich heavy metal binding proteins. In the mouse, MT-I and MT-II display a wide tissue distribution and have cysteine-rich heavy metal binding proteins. In the mouse, cytomegalovirus.

* This work was supported in part by Grant ES08089 from the NIEHS, National Institutes of Health (to J. A. J.), by a Burroughs Wellcome New Investigator in Toxicology Award (to J. A. J.), and by Grants ES05704 and CA61262 from the National Institutes of Health (to G. K. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by NIEHS, National Institutes of Health Toxicology Program Predoctoral Fellowship T32 ES07079.
¶ Supported by National Research Service Award Fellowship F32 ES05753.
** To whom correspondence should be addressed: Dept. of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160-7417. Tel.: 913-588-7517; Fax: 913-588-7501; E-mail: jkhaasok@kumc.edu.

1 The abbreviations used are: MT, metallothionein; mMT-I, mouse MT gene; MRE, metal response element; MTF-1, MRE-binding transcription factor-1; USF, upstream stimulatory factor; ARE, antioxidant responsive element; tBHQ, tert-butylhydroquinone; PKC, protein kinase C; EMSA, electrophoretic mobility shift assay; CMV, cytomegalovirus.

The Journal of Biological Chemistry Vol. 274, No. 9, Issue of February 26, pp. 5279–5284, 1999 Printed in U.S.A.
and $b$ buffer (100 mM KPO$_4$, pH 7.4, 4.0 mM ATP, 1.5 mM MgSO$_4$, 1.0 mM $Ca^2+$. Cells were lysed by freeze-thawing in 100 mM HCl. Cells were also treated with 0.4% ethanol or increasing doses of cadmium or zinc in 0.1 mM HCl. Cells were treated with 0.1 mM HCl or increasing doses of cadmium or zinc in 0.1 mM HCl. Cells were subsequently treated for 18–24 h. For dose-response studies, cells were treated with 0.1 mM HCl or increasing doses of cadmium or zinc. Cells were transiently transfected, phorbol 12-myristate 13-acetate in ethanol. In the continued presence of tBHQ in ethanol. To down-regulate protein kinase C (PKC), cells were transiently transfected, phorbol 12-myristate 13-acetate, cells were lysed in extraction buffer, and cell extracts were assayed for luciferase and $b$-galactosidase activity. The data are expressed as the luciferase/$b$-galactosidase ratio. Each value represents the mean ± S.E. ($n = 6$). Error bars not visible on the graph are covered by the symbol. $a$, significantly different from the corresponding control value ($p < 0.05$).

**Reporter Plasmids**—The proximal mMT-1 promoter fragments (~150 to +66 promoter (numbers relative to the transcription start point in the mMT-1 gene), ~150 to +66 promoter (deletion of ~100 to ~89), and the minimal ~42 to +66 promoter (9) were cloned into a luciferase reporter construct, pGL-2 basic (Promega Biotech, Madison, WI). Five tandem copies of the MREd (MREd$_5$) with the mMT-1 minimal promoter (~42 to +66) (9) were also subcloned into pGL-2 basic. The TATA box and transcription start site were provided by the mMT-1 promoter in these fusion genes. A single forward oriented copy of the USF/ARE (5'-GATCCGGGCGGCGGCGGCGACTATGGGCTGGGCTGGAA-3') was subcloned into the BglII site preceding the adenosine major late minimal promoter-luciferase reporter construct, pTi-luciferase, provided by Dr. William Fahl (University of Wisconsin, Madison, WI) (23). The bold portion of the sequence identifies the core ARE and USF binding site. In this construct the TATA box and transcription start site are provided by the adenosine major late minimal promoter. The mammalian expression vector CMV-mMTF-1 (24) was generated by inserting the mouse MTF-1 cDNA into the NotI site of a CMV expression vector kindly provided by Dr. James Smith (Baylor College of Medicine, Houston, TX).

**Transient Transfections**—IMR-32 human neuroblastoma or HepG2 human hepatoma cells were plated at a density of 6.0 × 10$^4$ cells/well in 24-well plates and grown in 0.5 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Transient transfections using the calcium phosphate method were done as described previously (25). IMR-32 and HepG2 cells were cotransfected 72 and 48 h after plating, respectively, with 430 ng of promoter-driven reporter plasmids and 20 ng of CMV-$b$-galactosidase to correct for transfection efficiency. Cells were incubated overnight with the transfection mix and then 100 µl of total protein was added to the EMSA buffer containing labeled MRE oligonucleotide, and the reactions were incubated on ice for 15 min. Reactions were subjected to polyacrylamide gel electrophoresis, the gel was dried, and the label was detected by autoradiography. The arrow indicates the MRE-mMTF-1 complex.

**Electrophoretic Mobility Shift Assay—EMSA**—Whole cell extracts were prepared as described previously (26) with modifications (8, 24, 27). IMR-32 human neuroblastoma cells or HepG2 mouse hepatoma cells were plated at a density of 3.0 × 10$^4$ cells in 100-mm dishes. IMR-32 cells were grown in 10 ml of Dulbecco's modified Eagle's medium. Transient Transfections—IMR-32 human neuroblastoma cells were plated at a density of 6.0 × 10$^4$ cells/well in 24-well plates and grown in 0.5 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Transient transfections using the calcium phosphate method were done as described previously (25). IMR-32 and HepG2 cells were cotransfected 72 and 48 h after plating, respectively, with 430 ng of promoter-driven reporter plasmids and 20 ng of CMV-$b$-galactosidase to correct for transfection efficiency. Cells were incubated overnight with the transfection mix and then 100 µl of total protein was added to the EMSA buffer containing labeled MRE oligonucleotide, and the reactions were incubated on ice for 15 min. Reactions were subjected to polyacrylamide gel electrophoresis, the gel was dried, and the label was detected by autoradiography. The arrow indicates the MRE-mMTF-1 complex.

![Graph](image-url)  
**Fig. 1. Transcriptional Activation of mMT-1 promoter in IMR-32 cells.** 72 h after plating, IMR-32 human neuroblastoma cells were transfected with a ~150 mMT-1-luciferase reporter construct and treated with increasing concentrations of tBHQ (A, △), cadmium (B, ■), and zinc (C, ◇). After 24 h, the medium was removed from the cells, the cells were lysed in extraction buffer, and cell extracts were assayed for luciferase and $b$-galactosidase activity. The data are expressed as the luciferase/$b$-galactosidase ratio. Each value represents the mean ± S.E. ($n = 6$). Error bars not visible on the graph are covered by the symbol. $a$, significantly different from the corresponding control value ($p < 0.05$).

![Graph](image-url)  
**Fig. 2. IMR-32 cell extracts do not contain MRE binding activity.** Electrophoretic mobility shift assays were performed with whole cell extracts from control, zinc-treated, cadmium-treated, and tBHQ-treated IMR-32 cells. Mouse HepG2 cells were treated with 100 µM zinc for the indicated time (h). IMR-32 cells were treated with 100 µM zinc, 1.0 µM cadmium, or 10 µM tBHQ for the indicated time (h). Whole cell extracts were made, 20 µg of total protein was added to the EMSA buffer containing labeled MRE oligonucleotide, and the reactions were incubated on ice for 15 min. Reactions were subjected to polyacrylamide gel electrophoresis, the gel was dried, and the label was detected by autoradiography. The arrow indicates the MRE-MTF-1 complex.
medium supplemented with 10% fetal bovine serum, and mouse Hepa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 2% fetal bovine serum. Protein concentration was determined using the BCA protein kit (Pierce). Whole cell extracts (20 μg of protein in 1 μl) or zinc-activated MTF-1 from an in vitro transcription/translation reaction (1 μl of a 50-μl reaction) were incubated in buffer containing 12 mM HEPES (pH 7.9), 60 mM KCl, 0.5 mM dithiothreitol, 12% glycerol, 5 mM MgCl₂, 0.2 μg of poly(dI-dC)/μg protein, 8 fmol of end-labeled double-stranded oligonucleotide (5000 cpm/fmol) in a total volume of 20 μl for 20 min on ice (8, 24). EMSA was performed using the oligonucleotide sequence MRE+ (5’-GATCCAGGGAGCTCAGCACGGGCCGAAAAGTA) (11). Protein-DNA complexes were separated at 4 °C using 4% polyacrylamide gel (acrylamide:bisacrylamide 80:1) electrophoresis at 15 V/cm. The gel was polymerized and run in buffer C (29). Protein-DNA complexes were separated at 4 °C using 4% polyacrylamide gel (acrylamide:bisacrylamide 80:1) electrophoresis at 15 V/cm. The gel was polymerized and run in buffer consisting of 0.19 M glycine, 25 mM Tris (pH 8.5), 0.5 mM EDTA. After electrophoresis, the gel was dried, and labeled complexes were detected by autoradiography.

RESULTS

Transcriptional Activation of mMT-1 Promoter in IMR-32 Cells—IMR-32 human neuroblastoma cells were transfected with a −150 mMT-1-luciferase reporter construct and treated with tBHQ, cadmium, and zinc (Fig. 1). Cadmium activated the −150 mMT-1-luciferase reporter 20–25-fold at doses of 0.5–2.0 μM (Fig. 1B). In contrast, tBHQ did not change the luciferase expression (Fig. 1A), and zinc caused only a modest but statistically significant increase of 1.6–2.0-fold (Fig. 1C). Activation of the mMT-1 promoter by heavy metals and oxidative stress in Hepa cells has been attributed to interaction between MTF-1 and MRE (7–9). Thus, the absence of functional MTF-1 in IMR-32 cells could account for the lack of responsiveness to zinc and/or tBHQ.

The hypothesis that this human neuroblastoma cell line has no functional MTF-1 was evaluated by EMSAs on whole cell extracts from IMR-32 cells compared with mouse Hepa cells (Fig. 2). Whole cell extracts were prepared from vehicle-treated, 10 μM tBHQ-treated, 1.0 μM cadmium-treated, and 100 μM zinc-treated cells (2 and 8). IMR-32 cell extracts did not contain detectable MRE binding activity at either 2 h (Fig. 2, lanes 2–5) or 8 h (Fig. 2, lanes 6–9). As has been demonstrated previously (8, 24, 27), treating Hepa cells for 1 h with 100 μM zinc results in significant MRE binding activity due to MTF-1 activation (Fig. 2, lane 1). We have previously shown that addition of unlabelled MRE oligonucleotide competes for the MRE-MTF-1 complex (8) and that the MRE-MTF-1 complex is supershifted specifically by a polyclonal antibody raised against MTF-1 (28).

MRE-MTF-1 binding activity can be increased in vitro by addition of exogenous zinc to whole cell extracts from untreated cells (8, 24, 27). Incubation of IMR-32 extracts with zinc in vitro did not generate a MTF-1-MRE complex (Fig. 3, lane 6). In contrast, treatment of Hepa control cell extract with zinc in vitro resulted in a large increase in MRE binding activity (Fig. 3, lanes 5 and 9). A possible explanation for these results is that IMR-32 extracts contain an inhibitor of MTF-1 activation and subsequent MRE-MTF-1 complex formation. A combined whole cell extract was prepared from a mixture of IMR-32 and Hepa cells to test this possibility (Fig. 3, lanes 4 and 8). The IMR-32 cell extract had no effect on activation of MTF-1 in Hepa cell extract, implying that IMR-32 cells do not possess a constitutively expressed repressor of MTF-1 activation.

The MRE Not USF/ARE Mediates Induction of the mMT-1 Promoter by Cadmium—We have recently demonstrated that the USF/ARE from the mMT-1 promoter participates in cadmium-mediated induction of mMT-1 in Hepa cells and that this composite site does not contribute to induction of mMT-1 by zinc (13). IMR-32 cells were transfected with −150 mMT-1-luciferase, −150ΔUSF/ARE-luciferase, and USF/ARE-luciferase reporter constructs to determine the role of USF/ARE in cadmium-mediated activation of mMT-1 promoter (Table I). Deletion of the USF/ARE from the intact −150 mMT-1 promoter sequence had no effect on induction of luciferase by cadmium in IMR-32 cells. The −150 mMT-1-luciferase reporter and −150ΔUSF/ARE-luciferase were activated 21.5- and 20.3-

![Table I](image)

**Transcriptional activation of the MT promoter by cadmium is dependent on the MRE and independent of the USF/ARE**

IMR-32 cells were plated at a density of 6.0 × 10⁴ cells/well in 24-well plates and grown in 0.5 ml of Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. The cells were transiently transfected and subsequently treated for 24 h. The medium was removed, and cells were lysed in extraction buffer. Cell lysates were then assayed for luciferase and β-galactosidase activity. Each value represents the mean ± S.E. (n = 6–12). NS, not significant.

| DNA construct       | Luciferase/β-galactosidase ratio (×10⁻²) | Fold activation |
|---------------------|------------------------------------------|----------------|
|                     | Vehicle | tBHQ (10 μM) | Cadmium (1 μM) | Zinc (100 μM) | tBHQ | Cadmium | Zinc |
| −150 mMT-1          | 1.35 ± 0.05 | 1.20 ± 0.03*  | 28.6 ± 0.57*  | 2.02 ± 0.08*  | 0.90  | 21.5    | 1.52 |
| −150ΔUSF/ARE        | 0.59 ± 0.02 | 0.60 ± 0.02  | 12.9 ± 0.58*  | 1.22 ± 0.04*  | NS    | 20.3    | 2.07 |
| USF/ARE             | 0.70 ± 0.04 | 0.72 ± 0.05  | 13.6 ± 1.30*  | 0.89 ± 0.06*  | 0.64  | 30.2    | 1.98 |
| MREΔd               | 0.45 ± 0.03 | 0.29 ± 0.02*  | 13.6 ± 1.30*  | 0.89 ± 0.06*  | NS    | 20.3    | 2.07 |

*a* Significantly different from the corresponding vehicle-treated cells (p < 0.05).
fold, respectively (Table I). In addition, the isolated USF/ARE element in a heterologous promoter construct was not activated by cadmium (Table I). Because the IMR-32 cells apparently do not have functional MTF-1, the MREδ5-luciferase was included to serve as a negative control. Contrary to this assumption, the data showed that MREδ5-luciferase was activated 30.2-fold by treatment with 1.0 μM cadmium (Table I). Thus, cadmium-mediated transcriptional activation of mMTF-1 promoter is MRE-dependent, yet MTF-1-independent in IMR-32 neuroblastoma cells.

**Overexpression of mMTF-1 Restores Zinc-mediated Induction of the MRE—**IMR-32 cells were cotransfected with increasing concentrations of a mammalian expression vector for mMTF-1 (CMV-mMTF-1) and the MREδ5-luciferase reporter construct (Fig. 4). Basal level expression of the MRE-driven fusion construct was significantly increased in a dose-dependent manner with increasing concentrations of CMV-mMTF-1 (Fig. 4A). At a concentration of 20 ng of CMV-mMTF-1/well there was a 9-fold increase in basal level transcription of the MRE. In addition, overexpression of MTF-1 regenerated a zinc-mediated induction of the MREδ5-luciferase reporter gene (Fig. 4B). The magnitude of zinc induction was 9-fold and maximal at 1 ng of CMV-mMTF-1. Notably, overexpression of mMTF-1 had no effect on activation of MREδ5-luciferase by cadmium and actually significantly decreased the extent of activation with increasing doses of CMV-mMTF-1 (Fig. 4B).

**MREδ5-luciferase Is Activated by Zinc in Human Hepatoma Cells Not Human Neuroblastoma Cells—**To validate that the observed effects in IMR-32 cells were not due to differences between rodent and human MTF-1, we transfected HepG2 human hepatoma cells with MREδ5-luciferase and treated them with zinc and cadmium. Zinc and cadmium transcriptionally activated the MREδ5-driven luciferase expression by 17- and 30-fold, respectively, in HepG2 human hepatoma cells (Fig. 5). Thus, HepG2 cells express functional human MTF-1 that is activated by zinc, whereas this transcription factor is apparently not functional or not expressed in IMR-32 cells. Based on these data, we wanted to determine whether activation of the MRE in IMR-32 cells was cadmium-specific. In addition to cadmium, we tested copper sulfate (5–200 μM), sodium arsenite (5–200 μM), sodium dichromate (0.1–20 μM), cobalt chloride (5–200 μM), nickel chloride (5–200 μM), lead acetate (0.1–50 μM), mercuric chloride (0.5–20 μM), manganese chloride (0.5–25 μM), bismuth nitrate (1–200 μM), and ferric chloride (5–200 μM). IMR-32 cells transfected with −150 MMT-1-luciferase or MREδ5-luciferase were treated with the above compounds, but only cadmium caused a significant increase in luciferase expression (data not shown).

**MREδ5-luciferase Activation in IMR-32 Cells Is PKC-independent**—Recently, Yu and co-workers (29) showed that metal-induced MT gene expression can be inactivated by PKC inhibitors. IMR-32 cells and HepG2 cells were PKC down-regulated by chronic treatment with phorbol 12-myristate 13-acetate, transfected with MREδ5-luciferase reporter, and treated with zinc and cadmium (Table II). In HepG2 cells, cadmium-mediated activation of the MRE was completely blocked by PKC down-regulation, and zinc-mediated activation was significantly reduced from 20.5-fold in control cells to 3.12-fold in PKC down-regulated cells. In contrast, PKC down-regulation in the IMR-32 cells had no effect on activation of the MRE by cadmium (Table II). Similarly, pretreatment of IMR-32 cells with the PKC inhibitors H7 and GF109203X had no effect on cadmium-mediated activation of the MRE (data not shown).

**FIG. 4.** Overexpression of mMTF-1 increases basal level expression and restores zinc-mediated activation of MREδ5-luciferase. 72 h after plating, IMR-32 human neuroblastoma cells were transiently transfected with a MREδ5-luciferase reporter construct and increasing amounts of CMV-mMTF-1 mammalian expression vector. The effect of mMTF-1 on basal level expression (A, ○) and vehicle-treated (○), 10 μM tBHQ-treated (▲), 1.0 μM cadmium-treated (■) and 100 μM zinc-treated (●) IMR-32 cells (B) was determined. After 24 h, medium was removed from the cells, the cells were lysed in extraction buffer, and cell extracts were assayed for luciferase and β-galactosidase activity. The data are expressed as the ratio of luciferase to β-galactosidase activities (A) and fold activation versus the corresponding control value (B). Each value represents the mean ± S.E. (n = 6). Error bars not visible on the graph are covered by the symbol. a, significantly different from the corresponding value in the absence of CMV-mMTF-1 (p < 0.05); b, significantly different from the corresponding vehicle-treated value (p < 0.05); c, significantly different from zinc-treated value in the presence of 1 ng of CMV-mMTF-1/well; d, significantly different from cadmium-treated value in the absence of CMV-mMTF-1.

**DISCUSSION**

These data demonstrate that zinc and cadmium have independent mechanisms for activation of the mMTF-1 promoter in IMR-32 cells. Because cotransfection of the mMTF-1 reporter constructs with a mammalian expression vector containing mMTF-1 restored zinc responsiveness to the IMR-32 cells, the lack of a zinc effect can be attributed to absence of functional
MTF-1 in this cell type. Interestingly, cadmium-driven activation of the MRE was not dependent upon the presence of functional MTF-1 and was actually attenuated by increased overexpression of MTF-1. Finally, this MTF-1-independent mechanism was specific for cadmium and not mediated through activation of PKC in IMR-32 cells.

In sharp contrast to our findings in IMR-32 cells, MTF-1, a zinc finger transcription factor, has been shown to be essential in mediating transcriptional activation of MT genes by zinc and cadmium in embryonic stem cells and BHK cells (12, 30). These transition metals are the most potent activators of mouse MT expression in many cell types and are proposed to converge on the MRE through MTF-1 activation. Furthermore, expression of MT-I and MT-II genes in MTF-1 null embryonic stem cells could not be increased by treatment with zinc, cadmium, copper, nickel, or lead, and there was no detectable basal level expression of either MT gene (12). These data suggest that MTF-1-MRE complex formation is essential for basal expression and induction of MT genes.

Zinc can reversibly and directly activate the DNA binding activity of MTF-1 (24), and the extent of MTF-1 binding activity is rapidly increased, as is occupancy of MRE in the mMT-I promoter, after treatment of cells with zinc (8). In contrast to zinc (8, 24), cadmium has little effect on the amount of MTF-1 binding activity in Hepa or HeLa cell extracts (27). Similarly, the DNA binding activity of recombinant human and mouse MTF-1 to MRE in vitro is also dependent on zinc and not facilitated by cadmium (27). These data support the hypothesis that cadmium may be activating MT genes by a mechanism distinct from that of zinc.

Fortuitously, we found that zinc did not transcriptionally activate mMT-1-luciferase reporter constructs in IMR-32 human neuroblastoma. But as was seen in the MTF-1 null embryonic stem cells (12), transfection of recombinant mMTF-1 into IMR-32 cells restored the zinc-mediated activation of mMT-1 reporters and increased their basal level expression. Indeed, it would appear that increased expression of MTF-1 actually attenuated transcriptional activation of the MRE by cadmium and zinc (Fig. 4B). Because both zinc and cadmium are affected and there is a corresponding increase in basal level expression of the luciferase reporter (Fig. 4A), we hypothesize that the greater the basal level expression (increased occupancy of the MRE by MTF-1) the lesser the induction of the MRE-luciferase reporter. This can certainly explain the effect of MTF-1 overexpression on zinc activation but also implies that activation of the MRE by cadmium can be competed for by MTF-1, suggesting that MTF-1 can effectively block the interaction of cadmium-responsive transcription factor(s) with the MRE. The mechanism(s) by which cadmium activates the MRE in IMR-32 cells remains to be determined.

The MRE may also bind the basal transcription factor Sp1 and zinc-regulated factor (31). We examined Sp1 by transfecting IMR-32 cells with a heterologous promoter construct containing the Sp1 consensus DNA binding site. This Sp1-luciferase reporter construct was not activated by cadmium, zinc, or tBHQ in the IMR-32 cells (data not shown), suggesting that the binding of Sp1 is not involved in activation of the MRE by cadmium in IMR-32 cells. The zinc-regulated factor is distinct from MTF-1 and was isolated from the mouse for its ability to activate MRE-driven reporter gene expression in yeast (31). A human homolog of the zinc-regulated factor has not been described, and the effects of cadmium versus zinc on its MRE binding activity has not been examined.

Yu and co-workers (29) demonstrated that metal-induced MT gene expression was blocked by inhibitors of PKC. The relatively nonspecific inhibitor of PKC, H7, blocked induction of MT by cadmium and zinc. The effect on zinc-mediated induction was due to a lack of uptake. H7 blocked the cellular accumulation of zinc. In contrast, the cellular accumulation of cadmium was unaffected by H7 pretreatment, suggesting that...
PKC could play an important role in regulating induction of MT by cadmium. We clearly show that PKC is not involved in the cadmium-mediated activation of MT reporters in IMR-32 cells. However, in HepG2 cells, the transcriptional activation of MRE reporter by cadmium is completely blocked by PKC down-regulation. Therefore, PKC is important in HepG2 cells but not in IMR-32 cells with respect to cadmium-mediated induction of MT gene expression. Alternatively, cadmium can stimulate myosin light chain kinase (32), affect calmodulin activity in the brain (33), and evoke inositol polyphosphate formation (34) and superoxide anion production by macrophages (35). The possible role for these pathways in mediating cadmium-driven MRE activation in IMR-32 cells has not been evaluated.

Others have reported differential gene activation in response to treatment with zinc versus cadmium (36). These investigators demonstrate that in HeLa-derived cadmium-resistant cells, cadmium but not zinc increases Hsp70 and GRP78 expression. In addition, a cis-acting element responsible for cadmium-mediated induction of human heme oxygenase in HeLa cells has been identified (cadmium response element) (37, 38). The core sequence is different from the MRE. EMSAs show that a protein constitutively associates with the cadmium response element, the DNA binding activity is not altered by cadmium, and the cadmium response element binding protein does not bind to the MRE. Furthermore, cadmium response element-luciferase reporters are activated by cadmium but not zinc, whereas MRE-luciferase reporters are activated by both cadmium and zinc in HeLa cells (38). Thus, it is obvious that cadmium and zinc can activate different genes through specific pathways/response elements. The clear separation of cadmium and zinc pathways for MT induction in IMR-32 neuroblastoma cells indicates that MRE activation by cadmium is not only independent of MTF-1 but also of zinc.

Targeted disruption of the MTF-1 locus is lethal to embryos at embryonic day 14 (39). The MTF-1 null embryos are devoid of MT-I and MT-II expression and have significantly reduced basal levels of γ-glutamylcysteine synthetase but normal expression of Sp1. Primary cultures of mouse embryo fibroblasts and established mouse embryo fibroblast cells lines from the MTF-1 null embryos were shown to have increased susceptibility to cadmium-induced cytotoxicity (39). The effect of cadmium on expression of MT genes in these MTF-1 null mouse embryo fibroblasts was not evaluated. Based on our data, there is an alternative mechanism by which to activate the MRE and MT gene expression independent of MTF-1 expression. MTF-1-independent activation of the MRE may be cell type-specific, and thus it is very important to determine whether cadmium increases expression of MT genes in MTF-1 null mouse embryo fibroblasts. Comparison of these data with the data generated in IMR-32 cells should help us begin to sort out the mechanism of activation by cadmium versus zinc. In conclusion, the data presented herein reveal a novel pathway for the induction of MT gene expression and MRE activation by cadmium. The relationship of these data to the regulation of MTs and other MRE-driven genes in vivo remains open to speculation.

REFERENCES
1. Kagi, J. H. (1991) Methods Enzymol. 205, 613–626
2. Masters, B. A., Kelly, E. J., Quaife, C. J., Brinster, R. L., and Palmiter, R. D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 584–588
3. Michalska, A. E., and Choo, K. H. (1995) Proc. Natl. Acad. Sci. U. S. A. 90, 8088–8092
4. Dalton, T., Fu, K., Palmiter, R. D., and Andrews, G. K. (1996) J. Nutr. 126, 825–831
5. Lazo, J. S., Kondo, Y., Dellapiazza, D., Michalska, A. E., Choo, K. H., and Pitt, B. R. (1995) J. Biol. Chem. 270, 5506–5510
6. Andrews, G. K. (1998) Prog. Food. Nutr. Sci. 14, 193–258
7. Stuart, G. W., Searle, P. F., Chen, H. Y., Brinster, R. L., and Palmiter, R. D. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7318–7322
8. Dalton, T. P., Li, Q., Bittel, D., Liang, L., and Andrews, G. K. (1996) J. Biol. Chem. 271, 26233–26241
9. Dalton, T., Palmiter, R. D., and Andrews, G. K. (1994) Nucleic Acids Res. 22, 5016–5023
10. Brugnera, E., Georgiev, O., Radtke, F., Heuchel, R., Baker, E., Sutherland, G. R., and Schaffner, W. (1994) Nucleic Acids Res. 22, 5167–5173
11. Radtke, F., Heuchel, R., Georgiev, O., Hergersberg, M., Gariglio, M., Dembic, Z., and Schaffner, W. (1993) EMBO J. 12, 1355–1362
12. Heuchel, R., Radtke, F., Georgiev, O., Stark, G., Aguet, M., and Schaffner, W. (1994) EMBO J. 13, 2873–2875
13. Li, Q., Hu, N., Daggett, M. A. F., Chu, W., Johnson, J. A., and Andrews, G. K. (1998) Nucleic Acids Res. 26, 5182–5189
14. Choudhuri, S., McKim, J. M., Jr., and Klaassen, C. D. (1993) Toxicol. Appl. Pharmacol. 119, 1–10
15. Rising, L., Vitarella, D., Kimelberg, H. K., and Aschner, M. (1995) Brain Res. 678, 91–98
16. Aschner, M. (1996) Neurotoxicology 17, 663–669
17. Kramer, K. K., Liu, J., Choudhuri, S., and Klaassen, C. D. (1996) Toxicol. Appl. Pharmacol. 136, 94–100
18. Kramer, K. K., Zoelle, J. T., and Klaassen, C. D. (1996) Toxicol. Appl. Pharmacol. 141, 1–7
19. Belloso, E., Hernandez, J., Giralt, M., Kille, P., and Hidalgo, J. (1996) Neuroendoecrinology 64, 430–439
20. Rojas, P., and Bies, C. (1997) Neurochem. Res. 22, 17–22
21. Zheng, H., Berman, N. E., and Klaassen, C. D. (1995) Neurosci. Int. 27, 45–58
22. Choudhuri, S., Kramer, K. K., Berman, N. E., Dalton, T. P., Andrews, G. K., and Klaassen, C. D. (1995) Toxicol. Appl. Pharmacol. 131, 144–154
23. Wasserman, S. W., and Fahl, W. E. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5361–5366
24. Johnson, J. A., and Nathanson, N. M. (1994) J. Biol. Chem. 269, 18856–18863
25. Dalton, T. P., Bittel, D., and Andrews, G. K. (1997) Mol. Cell. Biol. 17, 2781–2789
26. Zimarino, V., and Wu, C. (1987) Nature 327, 727–730
27. Bittel, D., Dalton, T., Ramson, S. L., Gedamu, L., and Andrews, G. K. (1998) J. Biol. Chem. 273, 7127–7133
28. Lee, D. K., Carrasco, J., Hidalgo, J., and Andrews, G. K. (1998) Biochem. J. 337, 59–65
29. Yu, C. W., Chen, J. H., and Lin, L. Y. (1997) FEBS Lett. 420, 69–73
30. Palmiter, R. D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1219–1223
31. Remondelli, P., Moltedo, O., and Leone, A. (1997) FEBS Lett. 416, 254–258
32. Chau, S. H., Bu, C. H., and Cheung, W. Y. (1995) Arch. Toxicol. 69, 197–203
33. Vig, P. J., and Nath, R. (1991) Biochem. Int. 239, 27–34
34. Singh, P. K., Jones, M. M., Gale, G. B., Atkins, L. M., and Smith, A. B. (1989) Toxicol. Appl. Pharmacol. 97, 572–579
35. Amorese, M. A., Witz, G., Goldstein, B. D. (1982) Toxicol. Lett. 10, 133–138
36. Cigliano, S., Remondelli, P., Minichiello, L., Mollone, M. C., Martire, G., Bonatti, S., and Leone, A. (1996) Exp. Cell. Res. 226, 173–180
37. Takeda, K., Ishizawa, S., Satoh, M., Yoshida, T., and Shibahara, S. (1994) J. Biol. Chem. 269, 22858–22867
38. Takeda, K., Fujita, H., and Shibahara, S. (1995) Biochem. Biophys. Res. Commun. 207, 160–167
39. Gu¨ nes, C., Heuchel, R., Georgiev, O., Muller, K. H., Lichtlen, P., Bluthmann, H., Marino, S., Aguzzi, A., and Schaffner, W. (1998) EMBO J. 17, 2846–2854