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Differential Expression of Human Metallothionein Isoform I mRNA in Human Proximal Tubule Cells Exposed to Metals

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In contrast to the single metallothionein (MT)-1 gene of the mouse, the human MT-1 gene family is composed of seven active genes and six pseudogenes. In this study, the expression of mRNA representing the seven active human MT-1 genes was determined in cultured human proximal tubule (HPT) cells under basal conditions and after exposure to the metals Cd²⁺, Zn²⁺, Cu²⁺, Hg²⁺, Ag⁺, and Pb²⁺. Basal expression of MT-1X and MT-1E mRNA in HPT cells was similar to expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. In contrast, mRNAs representing the basal expression of MT-1A and MT-1F were a minor transcript in HPT cells. Treatment of HPT cells with Cd²⁺, Zn²⁺, or Cu²⁺ increased the levels of MT-1E and MT-1A mRNA, but not the levels of MT-1X or MT-1F mRNA. The increase in MT-1E mRNA appeared to be influenced mainly by exposure to the various metals, whereas the increase in MT-1A mRNA was influenced more by exposure to a metal concentration eliciting a loss of cell viability. Treatment of HPT cells with the metals Hg²⁺, Ag⁺, and Pb²⁺ was found to have no effect on the level of MT-1 mRNA at either sublethal or lethal concentrations. Using HPT cells as a model, these results suggest that new features of MT gene expression have been acquired in the human due to the duplication of the MT-1 gene. Key words: cadmium, copper, gene expression, heavy metals, lead, mercury, metallothionein, mRNA, proximal tubule, RT-PCR, reverse transcriptase-polymerase chain reaction, silver, zinc. Environ Health Perspect 106:825–832 (1998).


The extrapolation of environmental studies from animal models to humans is potentially compromised when areas of examination involve human gene families with increased organizational complexity. A prime example is the human metallothionein (MT) gene family, in which gene organization is more complex in humans than in commonly used model systems. In both mice and humans, there are four classes of similar MT proteins, designated MT-1 through 4, defined on the basis of small differences in sequence and charge characteristics (1–3). For both species, the MT-1 and MT-2 proteins exhibit a ubiquitous pattern of tissue expression, whereas expression of the MT-3 and MT-4 family members is highly restricted (3–5). However, the gene organizations underlying the expression of the protein isoforms are quite different. In the mouse, the genes encoding the four MT isoforms are single-copy genes, and no MT pseudogenes are known to exist. The mouse MT-1 and MT-2 genes are separated by approximately 6 kb on chromosome 8 and appear to be coordinately regulated with functionally equivalent protein products (3,6). The MT-3 and MT-4 genes are closely linked to but not coordinately regulated with the other MT genes on mouse chromosome 8 (4,5). In contrast to the four single genes of the mouse, the human MT gene family is represented by 10 functional and 7 nonfunctional genes located at 16q13 (4,5,7,8). The genes encoding the MT-2, 3, and 4 isoforms are similar in number to the rodent (there is an MT-2 processed pseudogene on chromosome 2), but the human MT-1 locus possesses numerous MT-1 isoforms that are not present in the mouse (7,8). The human MT-1 gene locus is composed of seven functional genes and six pseudogenes. Complete genomic sequences are available for the seven active genes: MT-1A (9), MT-1B (10), MT-1E, MT-1F, MT-1G (11,12), MT-1H, and MT-1X (8). The potential significance of this duplication event is underscored by the fact that the human MT-1 isoforms genes have been shown to exhibit unique expression profiles with examples of inducer-specific, tissue-specific, and development-specific regulation (9,13–17).

The MTs are widely recognized and accepted as a major weapon in the cell's armamentarium for protection against and recovery from environmental insult. They are a class of low molecular weight (M, = 6,000–7,000), cysteine-rich, inducible, intracellular stress proteins that are best known for their high affinity for binding heavy metals. They are believed to serve an important role in the homeostasis of essential metals such as Cd²⁺ or Cu²⁺ during growth and development as well as in the detoxification of heavy metals such as Cd²⁺ and Hg²⁺, rendering the MTs important mediators/attenuators of heavy metal-induced toxicity, particularly hepato- and nephrotoxicity (1,3,18–20). To begin to define the regulation of expression of the MT gene family in human tissues and cells exposed to metals, we have undertaken an analysis of the metal-induced expression of the MT gene family in cultures of human proximal tubule (HPT) cells. This cell culture system was chosen because the kidney, and the proximal tubule in particular, represent an organ and cell type that are critically affected by chronic Cd²⁺ exposure in both animals and humans (21–23). We report here the expression of mRNA for each active MT-1 isoform in HPT cells as a function of exposure to Cd²⁺, Zn²⁺, Cu²⁺, Ag⁺, Hg²⁺, and Pb²⁺ at both lethal and sublethal concentrations.

Materials and Methods

Cell culture, metal treatments, and viability determinations. Total RNA samples for the analysis of MT-1 isoforms expression were obtained from a previous study that examined the viability and MT protein accumulation of HPT cells when exposed to sublethal and lethal concentrations of Cd²⁺, Zn²⁺, Cu²⁺, Ag⁺, Hg²⁺, and Pb²⁺ (24). Stock cultures of HPT cells were grown in 75-cm² T-flasks with a serum-free growth medium and a collagen matrix using procedures recently detailed (25). For use in experimental protocols, the cells were subcultured at a 1:2 ratio, allowed to reach confluence (6 days following subculture), and then exposed to media containing the various concentrations of metals. Thereafter, the cells were fed every 3 days with media containing the various concentrations of metals. We chose metal concentrations so that over a 16-day time course there would be situations where a loss of cell viability occurred and situations where exposure elicited no loss of cell viability. Metal concentrations eliciting these various levels of toxicity were determined in preliminary experiments. The effect of the individual components of the serum-free medium on the level of metal needed to elicit HPT cell toxicity has not been determined. These concentrations were determined to be 9, 27, and 45 μM for Cd²⁺; 20, 40, and 60 μM for Hg²⁺; 50, 100, and 500 μM for Pb²⁺.

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Figure 1. Expression of MT-1A and glyceraldehyde 3-phosphate dehydrogenase (G3PDH) mRNA in three HPT cell isolates. Reverse transcriptase-polymerase chain reaction (RT-PCR) products were electrophoresed on 2% agarose gels containing 0.5 μg/ml ethidium bromide. (A) Full gel showing reaction products for MT-1A from one cell isolate sampled at 30, 35, and 40 PCR cycles. (B) Full gel showing reaction products for G3PDH from the same isolate sampled at 30, 35, and 40 PCR cycles. (C) Expression of MT-1A (cycle 40) and G3PDH (cycle 30) for three cell isolates.

RNA isolation and RT-PCR. Total RNA was isolated according to the protocol supplied with TRI REAGENT (Molecular Research Center, Inc., Cincinnati, OH) as described previously (24,27). The concentration and purity of the RNA samples were determined using spectrophotometer scan in the UV region and ethidium bromide (EtBr) visualization of intact 18S and 28S RNA bands following agarose gel electrophoresis. Total RNA (0.5 μg) was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) as described previously (24,27). The primers developed for analysis of each of the active MT genes have been previously described (27). The primers for mRNA analysis were upper 5’CTCGAAATGGACCCCAACT3’ and lower 5’AGCAAAACCGTGTCAGTAGTTC3’ yielding a 287-bp product for MT-1B; upper 5’GCTTGCATGCTC- CACTGTTG3’ and lower 5’CAGGGTTGTGACATCTT3’ yielding a 284-bp product for MT-1E; upper 5’AGTTCTCTGCCTGTTGC3’ and lower 5’ACATCTGGAGAAAGTGTGTC3’ yielding a 232-bp product for MT-1F; upper 5’TCTTCTGCCTGGGAAACTTAA3’ and lower 5’AGGGGTCAAGATGTAGC3’ yielding a 309-bp product for MT-1G; upper 5’TCTCTTCTCTCCTGTTG3’ and lower 5’GCAAATGCTGGAGTTTGTAG3’ yielding a 315-bp product for MT-1H; and upper 5’GCTTGCATTGCTCCACAA3’ and lower 5’AGCAAAACCGTGTCAGTAGTTC3’ yielding a 287-bp product for MT-1E; upper 5’GCTTGCATGCTC- CACTGTTG3’ and lower 5’CAGGGTTGTGACATCTT3’ yielding a 284-bp product for MT-1E; upper 5’AGTTCTCTGCCTGTTGC3’ and lower 5’ACATCTGGAGAAAGTGTGTC3’ yielding a 232-bp product for MT-1F; upper 5’TCTTCTGCCTGGGAAACTTAA3’ and lower 5’AGGGGTCAAGATGTAGC3’ yielding a 309-bp product for MT-1G; upper 5’TCTCTTCTCTCCTGTTG3’ and lower 5’GCAAATGCTGGAGTTTGTAG3’ yielding a 315-bp product for MT-1H; and upper 5’GCTTGCATTGCTCCACAA3’ and lower 5’AGCAAAACCGTGTCAGTAGTTC3’ yielding a 287-bp product for MT-1B; upper 5’GCTTGCATGCTC- CACTGTTG3’ and lower 5’CAGGGTTGTGACATCTT3’ yielding a 284-bp product for MT-1E; upper 5’AGTTCTCTGCCTGTTGC3’ and lower 5’ACATCTGGAGAAAGTGTGTC3’ yielding a 232-bp product for MT-1F; upper 5’TCTTCTGCCTGGGAAACTTAA3’ and lower 5’AGGGGTCAAGATGTAGC3’ yielding a 309-bp product for MT-1G; upper 5’TCTCTTCTCTCCTGTTG3’ and lower 5’GCAAATGCTGGAGTTTGTAG3’ yielding a 315-bp product for MT-1H; and upper 5’GCTTGCATTGCTCCACAA3’ and lower 5’AGCAAAACCGTGTCAGTAGTTC3’ yielding a 287-bp product for MT-1B; upper 5’GCTTGCATGCTC- CACTGTTG3’ and lower 5’CAGGGTTGTGACATCTT3’ yielding a 284-bp product for MT-1E; upper 5’AGTTCTCTGCCTGTTGC3’ and lower 5’ACATCTGGAGAAAGTGTGTC3’ yielding a 232-bp product for MT-1F; upper 5’TCTTCTGCCTGGGAAACTTAA3’ and lower 5’AGGGGTCAAGATGTAGC3’ yielding a 309-bp product for MT-1G; upper 5’TCTCTTCTCTCCTGTTG3’ and lower 5’GCAAATGCTGGAGTTTGTAG3’ yielding a 315-bp product for MT-1H; and upper 5’GCTTGCATTGCTCCACAA3’ and lower 5’AGCAAAACCGTGTCAGTAGTTC3’ yielding a 287-bp product for MT-1B; upper 5’GCTTGCATGCTC- CACTGTTG3’ and lower 5’CAGGGTTGTGACATCTT3’ yielding a 284-bp product for MT-1E; upper 5’AGTTCTCTGCCTGTTGC3’ and lower 5’ACATCTGGAGAAAGTGTGTC3’ yielding a 232-bp product for MT-1F; upper 5’TCTTCTGCCTGGGAAACTTAA3’ and lower 5’AGGGGTCAAGATGTAGC3’ yielding a 309-bp product for MT-1G; upper 5’TCTCTTCTCTCCTGTTG3’ and lower 5’GCAAATGCTGGAGTTTGTAG3’ yielding a 315-bp product for MT-1H; and upper 5’GCTTGCATTGCTCCACAA3’ and lower 5’AGCAAAACCGTGTCAGTAGTTC3’ yielding a 287-bp product for MT-1B; upper 5’GCTTGCATGCTC- CACTGTTG3’ and lower 5’CAGGGTTGTGACATCTT3’ yielding a 284-bp product for MT-1E;
was performed as described on RNA obtained on days 1, 4, 7, 10, 13, and 16 of cell culture. Reaction products were removed at 30 cycles.

and lower 5'GGGCACACTTTGGCA-
CACGC3' yielding a 151-bp product for
MT-1X. Primers for glyceraldehyde 3-phos-
phate dehydrogenase (G3PDH) mRNA
were obtained commercially (Clontech, Palo
Alto, CA). The thermocycler was pro-
grammed to cycle at 95°C for a 2-min initial
step, then 95°C for 15 sec and 68°C for 30
sec, with a final elongation step at 68°C for
7 min. Controls for each reaction included
a no-template control where water was added
instead of the RNA and a no-reverse tran-
scriptase control where water was added
instead of the enzyme. Samples were
removed at 30, 35, and 40 PCR cycles to
ensure that the reaction remained in the
linear region. The final PCR products were
electrophoresed on 2% agarose gels contain-
ing EtBr along with DNA markers.

Results

**MT-IA.** The initial experimental goal was
to define the basal level of MT-1A mRNA
expression in HPT cells not exposed to
metals. We used mRNA expression for the
housekeeping gene G3PDH as a compari-
sion for MT-1A mRNA expression. The
reactions were stopped and samples
removed for analysis at 30, 35, and 40 PCR
cycles. The results demonstrated that basal
expression of MT-1A mRNA was very low
in HPT cells, demonstrating only a faint
band or no band for the reaction product
following 40 PCR cycles for the 3 HPT cell
isolates (Fig. 1A, C). This is in contrast to
G3PDH, where the same total RNA input
demonstrated a convincing reaction prod-
uct corresponding to G3PDH mRNA at 30
PCR cycles (Fig. 1B, C). These findings
indicate that HPT cells have a low basal
expression of MT-1A mRNA.

The effect of Cd²⁺ on the expression of
MT-1A mRNA was also determined on the
three independent HPT cell isolates after 1,
4, 7, 10, 13, and 16 days of exposure. Three
concentrations of Cd²⁺ (9, 27, and 45 μM)
were used in the time course so that the cells
experienced both lethal and sublethal levels
of Cd²⁺ exposure (24). Expression of MT-
1A was increased in HPT cells as a result
of Cd²⁺ exposure, and this increase was most
pronounced at Cd²⁺ concentrations eliciting
a loss of HPT cell viability (Fig. 2). For iso-
late 1, exposure to 9 μM Cd²⁺ elicited no loss
of cell viability over 16 days of exposure,
and MT-1A mRNA levels (at 40 reaction cycles)
exceeded control levels, appearing to increase
early in the time course and then decrease
(Fig. 2A, upper panel). After exposure to
either 27 or 45 μM Cd²⁺, where a loss of cell
viability occurs late and early, respectively, in
the time course, MT-1A mRNA levels were
clearly increased over control values. This is
effectively illustrated at 35 reaction cycles,
where convincing product bands corre-
sponding to MT-1A mRNA are present for
Cd²⁺-treated cells, but totally absent in con-
trol cells (Fig. 2A, lower panel). The second
cell isolate was more susceptible to Cd²⁺
and demonstrated a loss of cell viability after
1 day of exposure to 27 μM Cd²⁺. At the lower
concentration of Cd²⁺ (9 μM), where cell
loss was minimal during the time course,
MT-1A mRNA levels were clearly elevated
over control values (Fig. 2B). As was the case
for the first isolate, at the lowest concen-	ration of Cd²⁺ MT-1A mRNA levels appeared
to increase early in the time course and
return to undetectable levels by day 16 of
exposure. The third cell isolate had a pattern
of susceptibility to Cd²⁺ comparable to the
first isolate and showed increased MT-1A
mRNA levels and a similar pattern of MT-
1A mRNA expression (Fig. 2C). These find-
ings demonstrate that exposure to Cd²⁺
results in a repeatable increase in the level
of MT-1A mRNA for three independent HPT
cell isolates and that this increase is most
pronounced at Cd²⁺ concentrations that
impact cell viability.

A single HPT cell isolate was used to deter-
mine if MT-1A mRNA accumulation is
increased after exposure to lethal and sub-
lethal concentrations of Zn²⁺, Cu²⁺, Hg²⁺,

Figure 3. Expression of MT-1A mRNA in isolate 1 exposed to Zn, Cu, Hg, Ag, and Pb. Reverse trans-
scriptase-polymerase chain reaction for MT-1A was performed as described on RNA obtained on
days 1, 4, 7, 10, 13, and 16 of metal exposure. Isolate 1 was exposed to the following metals at
the indicated concentrations: (A) Zn, (B) Cu, (C) Hg, (D) Ag, and (E) Pb. Reaction products were
removed at 40 cycles for all metals except for Zn, which was removed at 30 cycles. The expression
of glyceraldehyde 3-phosphate dehydrogenase was constant (data not shown).

Figure 4. Expression of MT-1E and glyceraldehyde 3-phosphate dehydrogenase (G3PDH) mRNA in three
HPT cell isolates. Reverse transcriptase-polymerase chain reaction for MT-1E and G3PDH mRNA was
performed as described on RNA obtained on days 1, 4, 7, 10, 13, and 16 of cell culture. Reaction products
were removed at 30 cycles.

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Ag\textsuperscript{2+}, and Pb\textsuperscript{2+}. HPT cells exposed to Zn\textsuperscript{2+} demonstrated an increased accumulation of MT-1A mRNA at all three concentrations compared to control (Fig. 3A). The increase in MT-1A mRNA correlated to the level of Zn\textsuperscript{2+} exposure and was most pronounced at the highest concentration, where a loss of cell viability occurred. Analysis of MT-1A mRNA accumulation in Cu\textsuperscript{2+}-exposed HPT cells demonstrated increased accumulation of MT-1A mRNA similar to cells exposed to either Zn\textsuperscript{2+} or Cd\textsuperscript{2+} (Fig. 3B). In contrast to the increased accumulation of MT-1A mRNA demonstrated for HPT cells exposed to Cd\textsuperscript{2+}, Zn\textsuperscript{2+}, and Cu\textsuperscript{2+}, there was no increase in the accumulation of MT-1A mRNA for HPT cells exposed to Hg\textsuperscript{2+}, Ag\textsuperscript{2+}, or Pb\textsuperscript{2+} (Fig. 3C–E). The lack of MT-1A accumulation was noted at the highest concentrations of Hg\textsuperscript{2+}, Ag\textsuperscript{2+}, and Pb\textsuperscript{2+} that were previously determined to elicit a loss of HPT cell viability (24). For the six metals assessed, these findings demonstrate that only exposure to Cd\textsuperscript{2+}, Zn\textsuperscript{2+}, or Cu\textsuperscript{2+} results in increased HPT cell accumulation of MT-1A mRNA.

**MT-1E.** The basal expression level of MT-1E mRNA was evaluated in three HPT cell isolates using the total RNA samples described above. Results demonstrated that basal expression of MT-1E mRNA was evident after 30 PCR cycles for each HPT cell isolate. Product intensities were comparable to those noted for the G3PDH housekeeping gene at an identical cycle number (Fig. 4). Although a qualitative observation, the relative intensities of the MT-1E mRNA reaction products varied among the cell isolates, with isolate 1 demonstrating a more intense reaction product at 30 reaction cycles. This is interesting because isolate 1 has been shown to be the most resistant to the lethal effects of Cd\textsuperscript{2+} exposure, followed by isolates 3 and 2 (24).

The effect of Cd\textsuperscript{2+} on the expression of MT-1E mRNA was also determined for the HPT cell isolates. Results showed increased expression of MT-1E for all three HPT cell isolates as a result of Cd\textsuperscript{2+} exposure (Fig. 5), as evidenced by the notable increase in MT-1E mRNA at the lowest levels of Cd\textsuperscript{2+} exposure that produced no loss of cell viability. The Cd\textsuperscript{2+}-induced increase in MT-1E was not concentration dependent. A single HPT cell isolate was used to demonstrate that exposure to both Zn\textsuperscript{2+} and Cu\textsuperscript{2+} elicited an increase in MT-1E mRNA (Fig. 6A, B). As was the case for MT-1E mRNA of Cd\textsuperscript{2+}-exposed cells, the increase in expression was demonstrated to be in evidence at the lowest levels of Zn\textsuperscript{2+} and Cu\textsuperscript{2+} exposure where there was no loss of HPT cell viability. There were only slight increases in MT-1E mRNA accumulation as dosage levels of Zn\textsuperscript{2+} and Cu\textsuperscript{2+} were increased. The level of MT-1E mRNA was not increased over control when cells were exposed to Hg\textsuperscript{2+}, Ag\textsuperscript{2+}, or Pb\textsuperscript{2+} at both lethal and sublethal concentrations (Fig. 6C–E). In fact, there appears to be a trend for a small decrease in MT-1E mRNA for cells exposed to Hg\textsuperscript{2+}, Ag\textsuperscript{2+}, and Pb\textsuperscript{2+}. These findings indicate that MT-1E mRNA is a common transcript in HPT cells and that accumulation is induced by both lethal and sublethal exposure to Cd\textsuperscript{2+}, Zn\textsuperscript{2+}, and Cu\textsuperscript{2+}, but not by exposure to Hg\textsuperscript{2+}, Ag\textsuperscript{2+}, or Pb\textsuperscript{2+}.

**MT-1F.** The basal level of MT-1F mRNA expression was also assessed in three three HPT cell isolates. MT-1F mRNA, like MT-1A mRNA, was shown to be a rare transcript in HPT cells, having only minimal basal expression in two of the three cell isolates at 40 reaction cycles (Fig. 7). This is in contrast to G3PDH, where the same total RNA input demonstrated a convincing reaction product corresponding to G3PDH mRNA at 30 PCR.
cycles. In contrast to the results for MT-1A and 1E mRNA expression, exposure to Cd²⁺, Zn²⁺, and Cu²⁺ at both lethal and sublethal concentrations did not produce any repeatable or consistent increase in the accumulation of MT-1F mRNA (Figs. 8 and 9). There was also no increase in the level of MT-1F mRNA when HPT cells were exposed to Hg²⁺, Ag²⁺, or Pb²⁺ (Fig. 9C-E). These results indicate that MT-1F mRNA is a rare transcript in HPT cells under basal conditions and that the level of expression is not increased due to metal exposure.

**MT-1X.** Basal expression of MT-1X mRNA was prominent and similar to expression for G3PDH for each HPT cell isolate (Fig. 10). The effect of Cd²⁺ on MT-1X mRNA expression was also determined on three HPT cell isolates, and the results revealed only a marginal increase in MT-1X mRNA as a result of Cd²⁺ exposure (Fig. 11). This finding was reinforced by the levels of MT-1X mRNA in HPT cells exposed to both Zn²⁺ and Cu²⁺, where results suggested a slight elevation in MT-1X mRNA level following metal treatment (Fig. 12A, B). However, in no instance was the increase of the magnitude found for MT-1A and MT-1E mRNA under identical conditions. There was no increase in the level of MT-1X mRNA when HPT cells were exposed to Hg²⁺, Ag²⁺, or Pb²⁺ (Fig. 12C-E). These results demonstrate that MT-1X mRNA is a common transcript in HPT cells and that the level of expression is not greatly influenced by metal exposure.

**MT-1B, MT-1G, and MT-1H.** Basal expression and metal-induced expression of mRNA for the MT-1B, MT-1G, and MT-1H genes were also determined using the above RNA samples. No expression of MT-1B mRNA was demonstrated under basal conditions or after treatment with any of the six metals (data not shown). MT-1G and MT-1H mRNA displayed marginal bands approximately 20% of the time at 40 reaction cycles; however, the occurrence of bands had no discernible pattern under basal conditions or when the cells were exposed to the six metals (data not shown). We conclude that the expression of MT-1B, MT-1G, and MT-1H mRNAs is at the limit of detection of the RT-PCR assay and that repeatable data regarding expression cannot be obtained.

**Discussion**

One goal of this study was to determine if HPT cell cultures retained features of MT gene expression known to be present in the proximal tubule of the human kidney. Several studies using immunohistochemical analysis have shown that MT protein in the human kidney is localized exclusively in the proximal tubule (27-29). An analysis of MT mRNA expression in fetal and adult human kidney demonstrated the expression of mRNA representing the MT-2A, MT-1A, MT-1E, MT-1F, MT-1X, and MT-3 genes (27,30). For HPT cells, the present study demonstrated basal expression of MT mRNA for the MT-1A, MT-1E, MT-1F, and MT-1X genes. Earlier studies using HPT cells also demonstrated basal expression of MT protein and mRNA for the MT-2A and MT-3 gene (24,30). Basal levels of MT mRNA expression appear to be similar between the human kidney and cultured HPT cells. In total RNA preparation from human kidney tissue, mRNAs for the MT-2A, MT-1X, MT-1E, and MT-1F genes were demonstrated to be common transcripts, having expression levels close to the G3PDH housekeeping gene, while mRNAs for the MT-1A and MT-3 genes were demonstrated to be far less abundant (27,30). In the present study, basal expression of MT-1X and MT-1E mRNA was similar to expression of G3PDH mRNA in HPT cells, whereas MT-1A and MT-1F were less common transcripts. Earlier studies have shown MT-2A to be a common basal transcript and MT-3 a rarer transcript in HPT cells (24,30). Comparison of the findings between the HPT cells and the human kidney demonstrates that HPT cells retain the expected patterns of MT mRNA expression.

In a previous study, exposure of HPT cells to Cd²⁺, Zn²⁺, or Cu²⁺ induced a rapid and sustained accumulation of MT protein over a 16-day time course (24). In this respect, HPT cells demonstrated an expected response to Cd²⁺ exposure according to the large database of animal model studies (1-3,19). However, it was also shown in the previous study that the increase in MT protein elicited by HPT cell exposure to Cd²⁺ was not accompanied by a corresponding increase in the amount of MT-2 mRNA; even though such an increase would be expected from previous studies in animal models. Prompted by these results, an additional goal of the present study was to determine if regulation of the MT-1 genes in HPT cells exposed to metals corresponds to results from animal studies. In the mouse it has been shown that the MT-1 and MT-2 genes are coordinate-ly regulated and that mRNA is induced by treatment with the heavy metal Cd²⁺ (1-3,6). In contrast to the increase in MT-1 mRNA predicted from mouse studies, the mRNA expression pattern of HPT cells exposed to
The expression of Zn, (B) was dehydrogenase and Pb. Reverse metal exposure. Metal following 1, 4, obtained products were obtained on days 1, 4, 7, 10, 13, and 16 of cell culture. Reactions were sampled at 30 cycles for G3PDH and 30 cycles for MT-1X.

Cd\textsuperscript{2+} was MT-1 isoform specific. HPT cells demonstrated no basal or Cd\textsuperscript{2+}-induced expression of mRNA for the MT-1B, MT-1G, and MT-1H genes. HPT cells displayed notable basal levels of both MT-1E and MT-1X mRNA; however, only MT-1E mRNA was increased by exposure to Cd\textsuperscript{2+}. HPT cells displayed low basal levels of mRNA for both the MT-1A and MT-1F genes, although only MT-1A mRNA was increased by exposure to Cd\textsuperscript{2+}. These results, in conjunction with those for MT-2 described previously (29), provide convincing evidence that new features of MT gene regulation have been acquired in the human proximal tubule due to the gene duplication event in humans.

The most obvious consequence of the increased complexity and change in regulation of the human MT-1 and MT-2 genes would be to provide greater redundancy in the functional MT genes. The importance of redundancy in the MT genes can be inferred from the rodent species, in which the MT-1 and MT-2 genes are coordinately regulated and, from all examinations to date, the resulting proteins appear to be functionally equivalent. Such duplicate genes would not be expected to remain active if both were not needed for enhanced survival of the cell. Using HPT cells exposed to Cd\textsuperscript{2+} as a model, the gene duplication event in the human would further increase the level of redundancy in the absolute number of the MT genes and also in the regulation of these genes. In HPT cells, the MT-2 and MT-1X genes were demonstrated to have basal mRNA expression levels similar to G3PDH and to be unaffected by Cd\textsuperscript{2+} treatment. The MT-1E gene was also demonstrated to have a similar level of basal mRNA expression, but expression increased after exposure to Cd\textsuperscript{2+}. Messenger RNA for the MT-1A gene was shown to be a minor transcript in the HPT cells under basal conditions, but increased significantly as a result of Cd\textsuperscript{2+} treatment that approached levels causing a loss of cell viability. Based on data obtained using HPT cells, the duplication event in human MT genes can be
interpreted as providing a separation in the regulatory responsibility of the MT genes. That is, one subset of the human MT gene family (MT-1X and MT-2A) is responsible for providing basal levels of MT mRNA expression to the cell, and another subset (MT-1E and MT-1A) is responsible for providing an increase in MT mRNA expression due to Cd²⁺ exposure.

Further elucidation of the significance of an increased number of MT genes in humans is limited by the fact that no methods currently exist to determine the expression levels of individual MT-1 and MT-2 isoform-specific proteins. Without this ability, it is not possible to determine the relative contributions of decreased MT protein degradation and increased transcription rates on the induction of MT protein by metals. The antibodies currently available are believed to interact with all MT-1 and MT-2 isoforms, but this has never been convincingly shown, as this individual purified MT proteins are not available for cross-reactivity profiles. Recent development of the MT-1 and MT-2 null mouse allows the isolation of cell lines containing no basal MT protein levels. These should be excellent recipients for transfection by expression vectors containing each human MT isoform-specific gene, allowing the generation of homogeneous MT-isoform specific mRNA and proteins. The availability of such standards would allow the development and testing of assays capable of determining the level and functional properties of each specific MT isoform protein.

REFERENCES AND NOTES


Figure 12. Expression of MT-1X mRNA in human proximal tubule cells exposed to Zn, Cu, Hg, Ag, and Pb. Reverse transcriptase-polymerase chain reaction for MT-1X was performed using RNA obtained on days 1, 4, 7, 10, 13, and 16 of metal exposure. Isoform 1 was exposed to the following metals at the indicated concentrations: (A) Zn, (B) Cu, (C) Hg, (D) Ag, and (E) Pb. Reaction products were removed at 30 cycles for all metals. The expression of glyceraldehyde 3-phosphate dehydrogenase was constant (data not shown).