Metallothionein Expression in Animals: A Physiological Perspective on Function[1]

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ABSTRACT An integration of knowledge concerning regulation of metallothionein expression with research on metallothionein's proposed functions is necessary to delineate how this metalloprotein affects cellular processes, especially zinc metabolism. Metallothionein expression is driven by a number of physiological mediators through several response elements in the metallothionein gene promoter. Cellular accumulation of metallothionein depends on both gene expression and protein degradation. Both depend largely on availability of cellular zinc derived from the dietary zinc supply. Metallothionein expression is related to zinc accumulation in certain organs. Evidence has been produced, which suggests that metallothionein could act in a number of biochemical processes. It may act in zinc trafficking and/or zinc donation to apoproteins, including zinc finger proteins that act in cellular signaling and transcriptional regulation. As a result, metallothionein expression may affect a number of cellular processes including gene expression, apoptosis, proliferation and differentiation. The ability of metallothionein to exchange other metals with zinc in these proteins may explain a role in metal toxicity. Similarly, mobilization of zinc from metallothionein by oxidative stresses may explain its proposed antioxidant function. Apparent good health of metallothionein-deficient mice argues against a critical biological role for metallothionein; however, expression may be critical in times of stress. J. Nutr. 130: 1085–1088, 2000.

KEY WORDS: • zinc • metallothionein • gene expression

Metallothionein (MT)[3] was discovered over four decades ago. Nevertheless, this small cysteine-rich protein that tenaciously binds and exchanges specific metal ions, particularly zinc, still lacks an unequivocally established biological function. This situation is curious because MT has been the direct focus of >2500 research papers since 1957, with an equal number related to the protein in some way. MT research has been chronicled in the proceedings of four international meetings (1–4). This brief review is an overview that emphasizes selected integrative studies and salient mechanistic studies for the purpose of providing a physiological perspective on the continuing efforts directed at defining metallothionein's role in biology.

The rich abundance of the nonessential metal cadmium in equine kidney led to isolation and characterization of metallothionein (5,6). However, in normal human liver, zinc is the predominant metal bound to MT (7). There are four known isoforms of MT. MT-1 and -2 isoforms have a ubiquitous tissue distribution with particular abundance in liver, pancreas, intestine and kidney, and are the focus of this review, whereas MT-3 and -4 are found principally in brain and skin (1–4). Tetrahedral binding for Zn(II) in two metal-binding clusters utilizes thiolate ligands provided by all 20 cysteines in the protein to bind 7 atoms of zinc per MT molecule (Zn_7 MT). Binding exhibits high thermodynamic stability (1–4). However, the high kinetic lability of the metal-thiolate bond (8) suggests that metal exchange is a feature of any biochemical function ascribed to the protein.

Expression regulated by zinc. Metallothionein induction by zinc is blocked in intact animals and cultured cells by prior treatment with agents that inhibit mRNA production (reviewed in 9). Tissue Zn accumulation correlates with de novo MT synthesis (10–12), suggesting that the protein functions in trafficking or processing of newly acquired cellular zinc. Plasma zinc concentrations are related to MT expression, further suggesting a linkage to cellular zinc homeostasis (11,12). MT mRNA is relatively short lived, with maximal levels found hours after zinc is administered (10). MT mRNA was found primarily in the free polysomal pool (13), suggesting an intracellular function for the protein. Elements in the 3′ untranslated region of the mRNA may direct it to the perinuclear cytoplasm and cytoskeletal-bound polysomes (14). Although MT has been found to bind copper during copper toxicosis, the majority of data suggests that, under normal postnatal situations of adequate nutrition, this micronutrient is not a primary determinant of MT expression (9,15). Furthermore, in vivo and in vitro evidence has shown that apo-MT (thionein) is highly susceptible to proteolysis, whereas zinc and particularly cadmium binding make MT resistant to proteolysis (16,17). Consequently, cellular MT turnover and accumulation are linked directly to zinc availability from intracellular pools.

Transcriptional regulation of the metallothionein gene by metals is conferred by metal response elements (MRE) in the metallothionein promoter (reviewed in 18,19). Metal occupancy of a transcription factor that binds specifically to the MRE sequences of DNA (20) provides the positive stimulus for transcription. The MRE-binding transcription factor 1 (MTF-1) is a multiple zinc finger protein (21). Band shift analyses suggest that MTF-1 binding to DNA is activated by zinc, but not other transition metals, possibly through occu-

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[3] Abbreviations used: apo-MT (thionein), metal-free metallothionein; ARE, antioxidant response element; GRE, glucocorticoid response element; GSH, glutathione; GSSG, oxidized glutathione; hMT, human metallothionein; IL-1, interleukin 1; IL-6, interleukin 6; KO, metallothionein knockout; MRE, metal response element; MT, metallothionein; MTF-1, MRE-binding transcription factor 1; TG, metallothionein transgenic; Zn-MT, zinc-containing metallothionein

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pancy of one specific zinc finger. The K_d of this site is such that MTF-1 may obtain zinc from an intracellular pool that reflects zinc status and provide a direct link between cellular zinc status and MT transcription. In fact, nuclear zinc is labeled rapidly in a dose-dependent fashion after ^{65}Zn-containing diets of varying zinc content are consumed (22). MT mRNA levels in rats exhibit a hyperbolic response to increasing dietary zinc intake but little change in response to dietary copper (23), which agrees with the observed selectivity of zinc-induced MTF-1 binding to an MRE sequence (21). The biphasic response of MT mRNA levels as a function of dietary zinc intake, with maximal expression at an intake of ~100 mg Zn/kg diet in rats (23), could relate to autoregulation of MT synthesis at higher zinc intake levels. Multiple binding factors may further modify these metal-regulated processes (18,19). Of particular interest is that MTF-1 gene null mutations result in embryonic lethality (24), whereas MT-1 and -2 knockout (KO) mice are viable (25,26).

Expression regulated by stress and hormones. A wide variety of stresses, ranging from physical trauma to microbial infection, induce MT in animals (reviewed in 6,9,19). Receiving earliest attention as regulators of MT expression, glucocorticoids are responsible in part for augmenting hepatocyte zinc pools during periods of such stimulation (9,27). Two glucocorticoid response element (GRE) sequences are 17 kb upstream in the 5′ flanking region of the mouse MT promoter (28). Other hormones and cytokines act through intracellular signaling mechanisms (e.g., protein kinases) to induce expression. The metallothionein promoter also has one or more copies of other elements that initiate or enhance transcription rates (18,19). These include sequences for Sp1, AP-1, and AP-2. cAMP regulation may be mediated through AP-2. Interleukin (IL)-1, cAMP and glucagon enhance hepatic MT mRNA levels and also to alter kinetics of zinc metabolism (9). In particular, some increase the rate of zinc exchange among metabolic compartments of the plasma, hepatocytes and bone marrow (29). They may also affect zinc-related control of hepatic carbohydrate metabolism (30). An antioxidant response element (ARE) has been identified and shown to activate transcription in vitro (18,19). The ARE mediates MT expression in response to reactive oxygen species and may act synergistically with MRE. Promoter-specific DNA methylation, an action associated with growth suppression in rapidly dividing cancer cells (31), or an influence of MT on zinc binding domain of the p53 tumor suppressor (32) may suppress MT expression in tumors independently of MTF or other mediators of expression. This may explain why MT levels appear abnormally low in malignant tissue (33). Correlations of tissue MT content and causes of morbidity/mortality have been advanced (2–4).

Metallothionein expression in humans. A family of at least 12 MT genes, including all four isoforms, is located on human chromosome 16 (34). The MT-2A isoform accounts for >80% of human metallothionein (hMT) expression, at least in some tissues (35). hMT expression patterns change markedly in response to dietary zinc intake. The response appears similar to that clearly demonstrated in rats (reviewed in 15). Erythrocyte MT was directly related to zinc intake over a considerable range, including an intake below the recommended dietary allowance (36,37). Monocyte MT mRNA levels increased upon consumption of a zinc supplement and responded more rapidly than MT protein in erythrocytes (38). These ELISA and reverse transcriptase-polymerase chain reaction data demonstrate the responsiveness of MT expression to zinc consumption levels in humans. These relationships, when fully characterized, may help in delineating the relationship between zinc intake and MT function in human health.

Function of metallothionein. A physiological perspective on the function of MT requires consideration of the following:

- MTs display sequence homology and identical metal binding geometry throughout the spectrum of evolution from single-cell eukaryotic organisms to humans;
- the K_ds for metal binding to MT are high; specifically zinc is kinetically labile and may maintain MT thiols in a reduced redox state;
- the magnitude of expression of MT isoforms varies among tissues;
- MT synthesis *and* degradation are both factors in determining steady-state cellular MT concentrations;
- zinc transporter activities may affect MT turnover rates through regulation of intracellular zinc pools;
- MT KO mice are in general good health suggesting that MT is not critical for normal development or reproduction;
- basal MT expression is low in wild-type and MT transgenic (TG) mice when provided adequate dietary zinc;
- MT may translocate to the nucleus during cell proliferation and differentiation; and
- MT expression is responsive to a plethora of agents, and frequently accompanies/produces changes in intracellular and extracellular zinc trafficking.

The putative functions for MT include intracellular metal metabolism and/or storage, metal donation to target apometalloproteins (particularly zinc finger proteins and enzymes), metal detoxification, and protection against oxidants and electrophiles. These are summarized in **Figure 1**. The latter may be evolutionary adaptations to broaden the functional activity of MT in specific organ systems of higher animals (1–4). Evidence for these functions originally came from traditional animal, cell culture and in vitro models. More recently, these studies have been complemented by studies using mouse models with targeted deletion or transgenic overexpression of MT genes (25,26,39). Despite such a range of approaches, a clear function within an integrative context has yet to emerge. As pointed out by Bremner (15), with so many proposed functions for MT, it is likely this unique protein has "some relatively basic functions."

MT most likely functions in the regulation of zinc metabolism. Elevations of dietary zinc elevate intestinal MT (22) and maximal intestinal Zn accumulation seem to depend on MT synthesis (11). KO mice accumulate less zinc in the distal gastrointestinal tract when fed a high zinc diet (40), whereas TG mice accumulate more (Davis et al. unpublished observations). However, intestinal Zn and MT levels decline in rats after high zinc diets are fed for several weeks, indicating that other regulatory mechanisms take over (41). In most studies, zinc absorption was inversely related to intestinal MT content after MT was induced by dietary or parenteral zinc, or by fasting (reviewed in 42). Experiments with TG and KO mice confirm that MT can alter the processing of zinc taken orally because serum zinc concentrations were inversely related to intestinal MT level in TG mice and KO mice after single oral doses of zinc (43,44). ^{65}Zn ingested with food was absorbed less effectively than an aqueous zinc dose in KO mice (44), perhaps due to solubility differentials or an adaptation of the KO mouse to guard against high influxes of zinc that are normally handled by MT. When zinc influx is large and more rapid, as with the aqueous zinc doses, these compensatory mechanisms may be overwhelmed.

MT is also involved in systemic zinc distribution and in cellular zinc accumulation. The response of MT expression in

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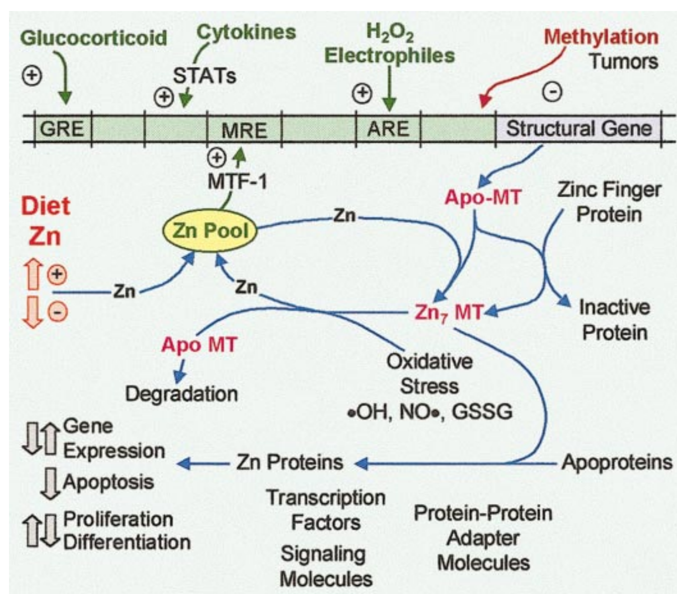

FIGURE 1 Overview of metallothionein (MT) gene regulation and function. The MT promoter has many response elements that up-regulate transcription. These include the following: *1*) metal response elements (MRE), which are activated by the metal-responsive transcription factor (MTF-1) after zinc occupancy, which is a function of the dietary zinc supply; *2*) glucocorticoid response elements (GRE); *3*) elements activated by STAT (signal transducers and activators of transcription) proteins through cytokine signaling; and *4*) the antioxidant (or electrophile) response element (ARE) activated in response to redox status. Methylation may down-regulate expression in some tumor cells. Cellular zinc pools are influenced by dietary zinc intake and zinc transporter activity, and serve as the source of zinc bound to MT. Zinc bound to MT exhibits high thermodynamic stability but also high kinetic lability. Apo MT (thionein) and Zn_7 MT (all coordination sites occupied) may serve to abstract or donate zinc, respectively, from/to zinc metalloproteins. Apo MT is more rapidly degraded than Zn_7 MT. The numerous zinc coordination sites of proteins (including transcription factors, signaling molecules and adapter molecules that use zinc fingers for protein-protein interaction) provide the opportunity for the cellular MT level to influence key processes, including gene regulation, cell proliferation and differentiation, signal transduction and apoptosis, as well as influence oxidative damage caused by oxidative stress and electrophiles.

animals to the administration of IL-1 and IL-6, interferon γ and other mediators has been documented (reviewed in 1–4,6,9). This is also true for endotoxin, which acts via initiating cytokines and other mediators. Cytokine-induced metallothionein expression is tissue specific. For example, both IL-6 and IL-1 will induce hepatic metallothionein in rats and mice (45,46), whereas isolated rat hepatocytes respond only to IL-6 (47), suggesting that IL-1 acts via stimulation of IL-6–producing cells in intact animals. The intricate array of response elements for physiologic mediators allows metallothionein expression to be amplified through the actions of multiple hormones and cytokines. Studies with KO mice and hepatocyte cultures from this genotype prove that hepatic zinc accumulation and transient hypozincemia due to treatment with specific cytokines and hormones depend on MT expression (48,49). Zinc accumulation during hepatotoxicity is also dependent on MT expression in TG and KO mice (Davis, unpublished data). MT expression also correlates with hepatic zinc accumulation during development, and protects against the embryotoxic and teratogenic effects of zinc deficiency during pregnancy in TG and KO mice (50–52).

MT may serve as a reservoir from which apometalloproteins, including enzymes and zinc finger proteins (transcription factors, signaling and adapter molecules), acquire zinc. Rapid exchange kinetics of zinc bound to MT, which is far greater than the exchange of zinc in other zinc proteins (8,53), supports this function. MT can successfully donate zinc to a number of zinc-dependent apometalloproteins in vitro. Reconstitution by MT rescues the enzymatic activity of some enzymes, including apocarbonic anhydrase and apocarboxypeptidase (53,54). Similarly, zinc from MT can be donated to some transcription factors (55). The exchange reaction may occur by direct donation of zinc from MT through a protein-protein interaction (53). Exchange with oxidized glutathione (GSSG) results in monophasic formation of a 1:1 Zn-glutathione (GSH) complex, which may also have a function in zinc mobilization from MT (56). GSSG also enhances the transfer rate of zinc from MT to apometalloproteins and increases the number of zinc atoms released (57). Release of cellular zinc from MT by GSSG would require a low GSH/GSSG ratio, a situation that occurs during oxidative stress. This implies redox control of zinc release from MT. A number of other biologically relevant disulfides and oxidants oxidize MT to disulfide-containing forms with concomitant release of zinc (58,59). These data imply that MT induction (by metals, oxidants and electrophiles) could regulate gene expression and cell proliferation by controlling occupancy of zinc binding sites in zinc finger transcription factors (32,55). Similarly, other zinc-sensitive processes, such as apoptosis, could be influenced by such cellular events (60).

The transfer of zinc from MT to other zinc metalloproteins is thermodynamically dependent on the K_d involved. Consequently, in some situations, zinc may be transferred from metalloproteins to apothionein. Several in vitro studies have shown that apothionein can remove zinc from zinc finger transcription factors. The result is loss of DNA binding activity, which is regained with free zinc or zinc-containing metallothionnein (Zn-MT) (61). Zn-MT rescues the function of cadmium-substituted tramtrack, a zinc finger transcription factor (55). When Cd displaces zinc in tramtrack, this protein loses DNA binding activity. Incubating Zn-MT with Cd-tramtrack in vitro allows exchange of cadmium and zinc, with the transcription factor regaining its DNA binding activity. Hence, Zn-MT may rescue zinc finger proteins from inactivation by other metals, explaining in part its proposed role in metal detoxification; in a broader context, the relative K_d of MT and the zinc finger protein determine the role of MT as a zinc donor.

In spite of the metal-donating properties of MT in vitro, questions remain about their in vivo implications. KO mice appear relatively normal during growth and development, and can handle a number of stresses including lipopolysaccharide treatment and high dietary or parenteral zinc loads. This argues against a critical role for MT in metal donation. On the other hand, MT transgenic mice exhibit altered zinc metabolism, which argues for a role in zinc trafficking. Further, redox-mediated zinc release from MT could explain the apparent protection against toxic metals, electrophiles and oxidative stress. It could be that metal donation (or removal) by MT from specific sites is most crucial during these stresses. Amplification of MT expression by hormones and cytokines supports such putative functions.

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