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MINIREVIEW

Metallothionein (42525A)

MICHAEL A. DUNN, T. LYNN BLALOCK, AND ROBERT J. COUSINS[1]

Food Science and Human Nutrition Department, and Center for Nutritional Sciences, University of Florida, Gainesville, Florida 32611

Metallothioneins (MTs) are low-molecular-weight cytosolic proteins found in eukaryotic species. MTs selectively bind heavy metal ions such as the nutritionally essential trace elements zinc (Zn) and copper (Cu) and the potentially toxic elements cadmium (Cd) and mercury (Hg). The biological functions of these proteins are not clearly resolved, but may include important roles in protection against and detoxification of heavy metals and, in the case of the nutrients Cu and Zn, regulation of their metabolism and perhaps function.

The metal ions which bind to MTs are involved in regulating the proteins' synthesis and degradation. To varying extents, heavy metals promote transcriptional activity of MT genes and the metal composition of the protein influences its rate of degradation. The metabolic fate of ions bound to MTs is also affected by the metal composition of the protein. Understanding these complex interrelationships between the metabolism of metal ions and MTs has been the thrust of much of the research done on MTs to date.

In addition to MT induction by metal ions, hepatic MT synthesis is also under hormonal control. This has fostered hypotheses that zinc metabolism can be regulated by hormonal signals that affect MT levels in tissues. This regulation may be an important part of the body's protective response to acute stress (such as infection) both by way of altering zinc distributions between tissues and within cells (hepatocytes, macrophages, and possibly others) and by virtue of recent evidence that MTs may be efficient scavengers of damaging free radicals.

After a brief, general introduction to MTs, much of this review focuses on the binding and turnover properties of MTs and how these may relate to biological function. Induction of MTs by hormones and stress and its possible biological significance is also emphasized. It should also be mentioned that, recently, molecular biologists have shown much interest in the structure and regulation of MT genes and the use of MT promoter sequences for genetic engineering experiments. Much of this research is beyond the scope of this review and is only mentioned when pertinent to the metabolism or functions of MTs. Detailed presentations of various aspects of MT are available (1–4).

Occurrence, Nomenclature, and Detection. Metallothioneins have been found in varying amounts in nearly all vertebrate tissues examined, with highest concentrations in liver, kidney, and intestine (see Refs. (1, 3, 5) for a list of species and tissues examined). MTs have also been reported to occur in invertebrates and microorganisms (1, 3). Some cell types within tissues (e.g., connective tissue cells, vascular endothelial cells, and some leucocytes) apparently lack MTs (5–7). Within cells MTs appear to be mainly cytoplasmic but immunochemical localization studies have shown that the protein is present within hepatic and renal nuclei as well (5, 6). Extracellularly, MTs have been found in low concentrations in plasma, urine, and bile (8, 9).

It is likely that the list of species and tissues in which MT-like proteins occur will continue to grow. It has therefore been recommended (1) that the term "metallothionein" be applied only to those proteins which have the following properties:

1. Low molecular weight (6000–7000 Da).
2. High content of heavy metals (7–12 metal atoms/molecule protein).
3. Optical properties characteristic of metal thiolates (mercaptides), i.e., metals bound only by clusters of thiolate bonds.

[1] To whom reprint requests should be addressed.

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4. An amino acid composition containing 23–33% cysteine with no disulfide bonds, aromatic amino acids, or histidines.

5. Amino acid sequence with conserved distribution of cysteinyl residues.

More specific terms such as Zn-metallothionein or Cd-metallothionein have been recommended for use when MT contains only one metal. The prefix used should not designate the metal used to induce the protein. When MT contains more than one metal, for example, 5-g atoms of Cd and 2-g atoms of Zn, terms such as (cadmium, zinc)-metallothionein or more specifically (Cd_5, Zn_2)-metallothionein should be used. In contexts where the metal composition is unknown or is of no special interest the term metallothionein alone is appropriate. The term "thionein" can be used to denote the metal-free protein.

Most vertebrate tissues contain two major "isoforms" of MT designated as MT-I and MT-II based on their elution position during ion-exchange chromatography. The MT-I isoform has been further resolved into subforms designated as MT-I_A, MT-I_B, etc. The relative proportions of the different isoforms of MT vary depending on species, tissue, physiological state, and exposure to metals. MT-II, however, is usually the predominant form.

Detection of MTs in tissues is most commonly done by isolating the protein via gel filtration chromatography and analyzing the eluant for metals by atomic absorption spectrophotometry. MTs characteristically elute at a V_e/V_0 ratio of about 1.8, a position where few other metal-binding proteins coelute. MT isoforms are usually detected by rechromatographing the MT fraction on anion-exchange columns and analyzing the eluant for metals. Recovery of MTs from ion-exchange columns is much less than 100%. Resolution of isoforms is improved with reversed-phase, high-performance liquid chromatography (10).

The amount of MT in tissues can be estimated using the techniques noted above by virtue of known molar ratios of metals to protein. The complete metal composition must be known, however, and care must be taken to prevent loss of metal from the protein. This is usually done by maintaining a pH above 7 and by including a reducing agent such as β-mercaptoethanol in all isolation steps since some reports indicate that oxidation of the protein can result in loss of metal and polymerization of the protein (11–14).

The MT content of tissues can also be determined by *in vitro* saturation of the binding sites of the protein with radioactive isotopes of either Cd, Hg, or Ag (15–17). The isotope used is determined by the binding strength of the metals being displaced from the native protein. Ag saturation is the only method that will displace copper from MT. Quantification is based on known molar ratios of metals to protein and the specific activity of the isotope used to saturate the protein.

Polyclonal antibodies against several mammalian MTs have been used in radioimmunoassays (RIA) to detect low levels of MT in tissues and plasma (18, 19). An enzyme-linked immunosorbent assay (ELISA) has also been developed (20). Recently the various methods for determination of MT in tissues have been compared (21). The RIA and Cd-saturation methods appeared to be best, overall.

Protein Structure and Metal Binding Sites. Comparison of all mammalian MTs studied reveals remarkably conserved structural features. All are single-chain polypeptides of 61 residues with *N*-acetylmethionine and alanine at the amino and carboxyl termini, respectively (1). The most unique aspects of the primary structure of MTs are the complete lack of aromatic amino acids and the high content of cysteine residues (up to 33%) which exceeds even that of the high-sulfur-containing keratins. The cysteinyl residues are rather uniformly distributed along the peptide chain in primarily cys–*x*–cys and cys–cys sequences. The amino acid replacements giving rise to the isoforms of MT are generally located outside these sequences and the positions of the cysteines as well as the amino acids adjacent to the cysteines are identical. The molecular weight of mammalian MTs determined from sequence data is about 6000 Da but can range from 6500 to 7000 Da depending on their metal composition. When determined by gel filtration their molecular weight is about 10,000 Da due to their prolate-related ellipsoid shape (1, 22).

The tertiary structure of MTs is characterized by two domains, an α domain within the

carboxy-terminal end of the molecule extending from amino acid 31 to 61 and an amino-terminal β domain extending from residue 1 to 30 (11, 23–25). Both domains are globular and linked by residues 30 and 31 to give the ellipsoidal shape to the overall molecule. Data from the crystalline protein have shown that in both domains, the polypeptide chain makes three reverse turns to spiral around metal ions (26). The β domain contains 9 cysteine residues and binds three atoms of zinc or cadmium or six atoms of copper. The α domain contains 11 cysteine residues and binds four atoms of zinc or cadmium or five to six atoms of copper (25). The metal ions are bound exclusively through thiolate coordination complexes which involve all 20 cysteine residues. Therefore the native protein contains no disulfide bonds or free sulfhydryl groups.

Both zinc and cadmium are bound in each domain in the 2+ valence state and are tetrahedrally coordinated to four cysteine thiolate ligands (18). The resulting complexes $[\text{metal}^{2+}(\text{cys}^-)_4]^{2-}$ are negatively charged and are thought to give rise to the overall negative charge of the protein. Copper, however, binds in the 1+ valence state. Coordination of copper binding is probably trigonal with three thiolate ligands involved (25). The relative affinities of heavy metal ions for binding to MTs differ. The stability constant for copper is about 100-fold greater than that for cadmium, which in turn is about 1000-fold greater than that for Zn (3). Both mercury and silver bind with greater affinity than even copper. Metal ions bound to MTs are released upon lowering the pH resulting in apometallothionein (thionein) (1). Metal reconstitution studies *in vitro* have shown that both zinc and cadmium preferentially fill the α domain first and then the β domain, whereas copper saturates the domains in the reverse order (11). Different metals then have preferred domains and the filling of domains *in vitro* is highly cooperative in that the binding of one ion in a domain promotes the binding of another in the same domain. This cooperative binding indicates that fully saturated domains are highly favored over partially filled domains.

The metal composition of native MTs isolated from tissues is seldom homogeneous, however. Even metal-induced MTs do not contain exclusively the metal used as an inducer (27). The structure and cooperative binding characteristics for mixed-metal MTs (especially copper-containing MTs) are not well understood. For example, it is not known whether Cu and Zn can be bound within the same domain, but *in vitro* studies have shown that saturation of the β domain with copper does not interfere with the normal binding of Cd to the α domain and vice versa (28). Also, stepwise displacement of Zn from Zn-saturated MTs with Cd showed that Cd binding in this situation was not cooperative and there was no preference for either domain (29). Furthermore, the resulting mixed-metal MTs did not resemble metal cluster compositions found in native (Cd_5, Zn_2)-MTs, indicating that native MTs are not formed by stepwise addition of Cd to Zn-MTs.

Native metal distributions were produced *in vitro,* however, by simply mixing together appropriate amounts of Cd_7-MT and Zn_7-MT (29). This demonstrates that direct intermolecular exchange of metals can produce mixed-metal MTs and that this may be the pathway used *in vivo.*

In the exchange studies just mentioned, Cd appeared to prefer the α domain and Zn the β domain. Thus, an apparent displacement effect is present when Cd_7-MT and Zn_7-MT are mixed together resulting in movement of Cd from the β region of Cd_7-MT to the α region of Zn_7-MT, thus displacing α Zn to the β domain. These phenomena are probably related to the steric properties of the two metal clusters and/or to the existence of site-specific exchange pathways between interchanging MTs (29). These metal exchange reactions also demonstrate that binding of metals to MTs is reversible and rapid. As will be discussed below, the metal exchange reactions for MT may have important physiological significance.

Synthesis, Degradation, and Metal Turnover. Synthesis of MT is induced to various extents by different metal ions, hormones, and other factors. The metabolic fate of MTs, including turnover of bound metals and degradation of the protein itself, is also variable and depends on the metal composition of the protein. These variable characteristics of protein and metal turnover may provide the basic mechanisms through which MTs exert their

biological functions. This concept is further illustrated in a later section dealing with the functions of MTs. In the present section the mechanisms of MT synthesis, degradation, and metal turnover are discussed.

Regulation of synthesis by metals. For many years it has been known that the MT content of liver, kidney, and intestine can be greatly increased by parenteral or dietary administration of Cd, Cu, or Zn. That increased MT synthesis was involved in this accumulation was first demonstrated by increased incorporation of [^{35}S]cysteine into the protein (30–34). Since those early observations, heavy metals have been shown to induce MT synthesis primarily by increasing the rate of MT gene transcription (reviewed in (2, 3)). For example, zinc, cadmium, copper, and mercury increase the transcription rate of the MT-I gene in mouse kidney and liver several hours before maximal accumulation of MT-I mRNA and maximal rates of MT biosynthesis occur (35). The various metals differed, however, in their effectiveness as inducers within a tissue as well as between tissues. In the liver, Cd and Zn were the best inducers. Copper was a good inducer only at high doses and induction by Hg was weak. In contrast, Hg ranked with Cd as the best inducer in kidney with Zn being a good inducer at high doses and Cu exhibiting only weak induction. At practical levels of dietary copper (1–36 mg/kg) and zinc (5–180 mg/kg) MT mRNA levels in liver, intestine, and kidney, but not brain, are increased by higher zinc intakes (36). This is particularly true when copper intake is low. These findings demonstrate that metal–tissue interactions are important in the regulation of MT synthesis. Possible explanations for these interactions include differences in (A) a tissue's ability to take up a particular metal from the systemic circulation, (B) the accessibility of metals to regulatory factors (proteins and metal regulatory elements) within cells, and (C) the transcriptional potential (inducibility) of MT genes within these cells.

Recent evidence indicates that the major isoforms of MT may also be induced to different extents by different metals and that this may be species and tissue dependent. To date, only the metals mentioned above (Cd, Zn, Cu, and Hg) and Ag, Au, Ni, Co, and Bi have been shown to regulate MT gene transcription (35, 37, 38). Some of these metals may act indirectly, however, by displacing Zn from MTs and then Zn acts as the direct inducer or via stress associated with administration of pharmacological doses of the metal.

The number of structural MT genes in mammals varies from two in the mouse (one for each isoform) to considerably more in primates (reviewed in (3, 4)). The number of metal regulatory elements (MRE) for each gene may also vary between species. MREs are upstream from the structural gene and contain closely related base sequences. Having multiple copies of metal regulatory sequences may allow various regulatory factors (possibly specific proteins) generated by different cell types to act either cooperatively or antagonistically with each other in terms of their induction of MT synthesis.

The molecular mechanisms by which metal ions interact with the MRE to induce transcription are not well understood. Binding proteins that bind both the MRE and the inducer metal are the most likely mode of action. Recently, in bacterial cells, a 16-kDa protein that was shown to bind to the promoter region of the gene conferring mercury resistance to these cells has been isolated. This potential, trans-acting regulatory protein is thought to act as a repressor in the absence of Hg and a positive regulator in the presence of Hg (39). Although MTs have been found to be associated with nuclei (5), speculation that thionein itself may be a regulatory protein seems unfounded based on the size of the regulatory protein mentioned above and on reports that MT fusion genes are normally inducible in cells that produce little or no thionein (see (3)).

Regulation of synthesis by hormones and other factors. Metallothionein synthesis is also increased by hormones including glucocorticoids, glucagon, and epinephrine and by a number of other factors including cAMP, interferon, and interleukin 1. A variety of acute stresses including food restriction, physical restraint, and tissue injury resulting from inflammatory agents or bacterial endotoxin have also been shown to induce MT synthesis (2).

Glucocorticoids injected into mice increase MT-I synthesis mainly in the liver (40). Glucocorticoids, glucagon, epinephrine, and dibutyryl cAMP (Bt_2cAMP) have all been shown to increase liver MT mRNA levels (41). These

effects appear to be produced primarily at the level of transcription since glucocorticoids have been shown to increase MT transcription rates in isolated mouse liver nuclei and the accumulation of MT mRNA in rat liver in response to glucagon, epinephrine, and Bt_2cAMP has been blocked by actinomycin D. All inducers of MT that are sensitive to actinomycin D have subsequently been shown to act via transcriptional regulation (2).

The molecular mechanisms by which glucocorticoids stimulate MT gene transcription are not well understood, but a glucocorticoid–receptor complex has been postulated to interact with MT promoter sequences. However, the MT promoter is not responsive to glucocorticoid hormone in fusion gene experiments (42).

The ability of Bt_2cAMP (a synthetic cAMP analog) to increase both MT proteins and MT mRNA levels in rat liver suggests that cAMP is an intracellular regulator of MT gene transcription after induction by glucagon or epinephrine. That cAMP is acting at the level of transcription seems probable since its effects are blocked by actinomycin D and, recently, it has been shown to increase the rate of MT gene transcription by isolated rat liver nuclei (43).

Interferon has also been shown to increase the rate of MT gene transcription through an, as yet, unknown mechanism. An interferon regulatory control sequence has been postulated (44). Therefore, it is of interest that stimulation by interferon of natural killer activity in lymphocytes from individuals at risk for AIDS is augmented by zinc (45). Induction of MT synthesis could be related to this enhancement in natural killer activity by zinc since MT inducers stabilize macrophages (46) and perhaps other cells.

MT synthesis is also induced by a variety of inflammatory agents and interleukin 1, a mediator of the acute phase response (reviewed in (2)). MT is also induced by inflammatory agents in adrenalectomized rats (47–49). Furthermore, fusion genes with the MT promoter are regulated in transgenic mice by endotoxin administration (50), suggesting inflammatory agents control MT gene transcription by a mechanism that is independent of either glucocorticoid or metal regulation. It has been postulated, however, that endotoxin and interleukin 1 induction is mediated or augmented by glucagon or other hormones (48, 51).

The mode of MT induction by food restriction and physical stress has not been well studied. Hormonal regulation of gluconeogenesis during fasting is complex and involves many signals that also act as MT-inducing agents during these situations (2). For example, actinomycin D blocked MT production in fasted rats suggesting that changes in MT mRNA levels are involved (52). Recent evidence suggests that stress-induced MT production may involve more than glucocorticoids or glucagon alone (53).

It should be pointed out that the above discussion of MT induction by metals and other factors centered on studies in which rather large acute doses of stimuli were used to induce MTs in order to study mechanisms of regulation. How these findings may relate to induction by dietary metals and normal physiological changes is mentioned in later sections.

Degradation and metal turnover. Conditions that affect the synthesis of MTs give important information concerning when MTs may be needed in tissues. Equally important in understanding the functions of MT, but less well understood, is the degradation of the protein and the turnover of metals bound to it. These processes, which are regulated in part by the metal composition of MT, may be basic determinants of the metabolic fate of the bound metals as well as of the protein itself.

The movement of metals through the cellular pool of MT molecules can be thought to occur in three ways: (A) release of bound metals concomitantly with the degradation of the protein; (B) exchange of bound metals with free metal ions and metals bound to specific ligands (including MT) without degradation of the protein; and (C) transfer of intact metal–protein complexes out of the cytoplasm into another cellular compartment, lysosomes, for example. Limited evidence suggests that all three of these processes can occur *in vivo* and may give rise to some of the multiple functions postulated for this protein and perhaps altered MT turnover associated with some diseases.

Estimates of the half-life of MTs, based on the disappearance of [^{35}S]cysteine from the labeled MT pool in hepatic cytosol, initially indicated that Cu-MT was degraded faster than

Zn-MT which was faster than Cd-MT (half-lives were 12–17, 18–20, and 84 hr, respectively) (54–56). The extent that the protein is actually degraded, however, suggests that thionein (apo-MT) is the most rapidly degraded, followed by Zn-MT and Cd-MT, with Cu-MT being resistant to degradation (57–59). Incubation of MTs *in vitro* with nonlysosomal proteases showed that trypsin degraded Zn-MT by 17% and Cd-MT by only 10% in 24 hr. Similarly, Pronase degraded both these MTs by only 15% in 24 hr. In contrast, lysosomal extracts degraded Zn-MT by 50% and Cd-MT by 17% in only 1 hr (57). Copper-MT, however, resists lysosomal degradation (58, 59). These findings suggest that protein degradation with release of bound metals occurs only to a limited extent in the cytosol but may readily occur in lysosomes, at least for zinc- and cadmium-containing MT.

The fate of zinc released by MT degradation is unknown. Cadmium is thought to reassociate with cytosolic MT since it can be found there for long periods (up to months) after the protein is induced by cadmium injection (55, 60, 61). Copper, however, has been found to accumulate in hepatic lysosomes under conditions associated with high liver copper levels, particularly Wilson's disease (62–65). Specifically, copper is present as insoluble aggregates of copper-MTs possibly formed by oxidation of thiol groups and intermolecular disulfide bond formation. The disappearance of [^{35}S]-cysteine from cytosolic copper-MTs *in vivo* then may not be a function of protein breakdown, but may reflect transfer of Cu-MTs into lysosomes.

Partial degradation of MTs is also possible. Proteolysis of Cd-MT with subtilisin results in an intact α domain but full degradation of the β domain (19). The relative stabilities of the two domains may then differ, depending on their metal composition. The metabolic fate of the metals bound to the two domains may also differ under *in vivo* conditions.

MTs may readily exchange metals with either free metals ions in the cytosol or ions bound to other ligands including other MT molecules. The half-life of ^{64}Cu bound to hepatic MT *in vivo* has been found to be less than 2 hr whereas the half-life of [^{35}S]cysteine incorporated into MT was 12 hr (66). The fact that radiocopper disappears from MT much faster than ^{35}S indicates that copper can be released from its binding sites without degradation or polymerization of the protein. Since the total copper content of the protein remains relatively constant, copper exchange is implied. Similar conclusions were drawn from studies with ^{65}Zn which showed that the ^{65}Zn content of hepatic MTs can fluctuate while the total zinc content of the protein remains constant (67). *In vitro* exchange studies between cadmium and zinc-MTs also demonstrate that both metals can rapidly (within minutes) interchange between MT molecules (29). The exchange of zinc observed in cultured hepatocytes suggests at least two metabolic pools, both exhibiting rapid kinetics (68). Both are expanded by glucocorticoids suggesting that MT induction is involved. In cultured Chinese hamster cells, uptake of ^{67}Cu and binding to MT were shown to be followed by a redistribution to higher-molecular-weight fractions, presumably copper metalloenzymes (69), suggesting that MT copper can be transferred to other ligands. The ability of MTs to donate metals to apometalloproteins reactivating them *in vitro* is further evidence that metal can be released from MTs at physiological pH's without protein degradation. This is discussed below.

The various aspects of MT synthesis and degradation are shown in Fig. 1.

Functions. It is generally accepted that the major functions of MT are related to metal metabolism although defining the exact nature of these functions remains difficult. Postulated functions include detoxification and storage of heavy metals and the regulation of cellular copper and zinc metabolism in response to dietary and physiological changes. Within the context of regulating copper and zinc metabolism are possible roles in both the control of fetal development and cellular adaptation to various types of stress. Other potentially important functions not directly related to controlling metal metabolism have been postulated. These include free radical scavenging and protection against uv and X-ray damage. The ubiquitous distribution of MT or MT-like proteins in both plant and animal phyla, including single-celled organisms, mandates that proposed functions be circumspect. With

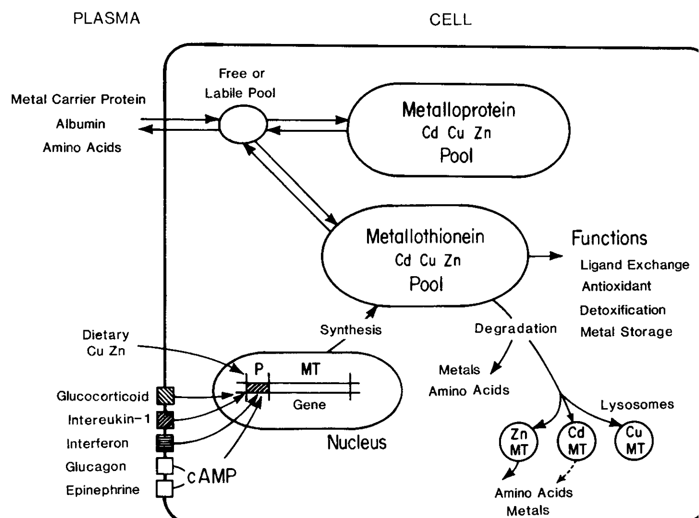

FIG. 1. Schematic diagram of proposed metabolism and functions of metallothionein. Metals carried in the plasma on proteins or amino acids exchange rapidly with a labile metal pool in the cytoplasm which in turn exchanges metals with MT and other metalloproteins. The size of the MT pool is regulated by MT synthesis and degradation. MT synthesis is regulated by dietary metals, hormones, and other factors which induce transcription of the MT gene via interaction with specific promoters (P) located upstream from the MT gene. cAMP may be the regulatory factor mediating induction by glucagon and epinephrine. Other regulatory factors that interact with the promoter are presumed, but remain unidentified. Degradation of MT may occur in the cytoplasm or within lysosomes. The rate of degradation varies with metal composition (Zn > Cd > Cu). Copper-MTs may accumulate within lysosomes.

the evolution of hormonal regulation divergent functional roles for MT may have developed.

One potentially powerful approach to studying the functions of MT is to select for, or genetically alter, cells so that they produce higher or lower than normal levels of the protein. Experiments using this approach support a role for MT in metal detoxification. For example, cultured mammalian cells that have lost their ability to make MTs (due to gene hypermethylation) are hypersensitive to cadmium toxicity (70). Conversely, cell lines with additional MT genes overproduce MTs and become highly resistant to cadmium (71). Furthermore, cells selected for cadmium resistance produce higher than normal levels of MTs due to gene amplification (72). Similar approaches indicate that MT also protects cells from copper, zinc, and mercury poisoning (37).

Limited evidence suggests that MTs also function to detoxify metals in intact animals. Under conditions associated with high-hepatic-copper levels, i.e., chronic high-copper intakes and inherited copper toxicosis, copper accumulates in a relatively nontoxic form within lysosomes as polymeric copper-MT (62, 63). The mechanism by which copper-MTs accumulate in this organelle is unknown, but is probably related to the limited proteolysis of copper-MT. Mammalian neonatal liver also contains a large amount of copper stored in this form (13, 63). Presumably this is used as a source of copper during the neonatal period since milk is a poor source of this nutrient. Other studies with intact animals report that induction of MTs by pretreatment with zinc or low doses of cadmium can protect the animal against subsequent lethal doses of cadmium (reviewed in (1)).

The known characteristics of cadmium-MT seem consistent with it being well suited for detoxifying this metal. MT is readily induced by and has a high affinity for cadmium. Furthermore, the stability of MTs against proteolysis is greatly increased after binding Cd and the degradative release of the metal results in

its reassociation with other MTs (55, 57). This cycle apparently can keep cadmium in a relatively nontoxic form within cells. It must be emphasized that the metal is still metabolically active and exhibits ligand exchange, albeit MT becomes the predominant ligand involved.

Not all metals which induce MTs are detoxified by these proteins. Cell lines which overproduce MTs by gene amplification do not show increased resistance to Ag, Au, Co, or Ni, even though these metals induce MT synthesis (37). It has been suggested that these metals either do not bind to MTs or produce unstable complexes with the protein that are rapidly degraded.

Because the synthesis and degradation of zinc and copper-MTs are subject to complex controls it seems likely that these proteins play an important role in cellular essential metal metabolism. MT could potentially modulate the movement of Cu and Zn within cells either directly through donation of these ions to metal requiring apoenzymes or metabolic compartments (such as membranes) or indirectly by regulating their free or available concentrations. Specific suggestions have been directed at a function of MT in increasing the available pool of intracellular zinc. The increase in zinc exchange that accompanies increased MT synthesis supports such a hypothesis (68, 73). This latter role is supported on a biophysical basis as well (74). Emergence early in evolution would be in concert with this role.

A function for MT in the regulation of intestinal absorption of copper, zinc, and other metals has been proposed (reviewed in (2)). There is a clear inverse relationship between intestinal MT levels and absorption of copper and zinc. As has been shown with other cell types, zinc exchange rates with MT in intestinal cells is rapid, which indicates that the protein could act as a dampening factor in absorption. The lethal-milk (lm) mutation in mice has been related to an overproduction of intestinal MT (75). Clinical signs of a systemic zinc deficiency develop. Genotypic expression of lm is reversible with zinc supplementation. Similarly, beneficial effects of zinc therapy in Wilson's disease patients which causes negative copper balance have been suggested to result from increased intestinal MT production and a concomitant decrease in copper absorption (76).

Several apoenzymes have been shown to be activated *in vitro* by Zn- and Cu-MTs (77–80). Activation experiments such as these must be interpreted with caution because an equilibrium would be established between any metal complex and an apoenzyme that could produce increases in enzyme activity. Such a function has not been demonstrated *in vivo.*

Dietary zinc intake correlates positively with tissue levels of MT mRNA and MT-bound zinc. For example, increasing the dietary zinc intake from 5 to 30 mg/kg produces increases in MT mRNA levels in liver, kidney, and intestine (36). Dietary copper intake levels do not markedly influence MT mRNA levels under the same conditions. Zn repletion studies have shown levels of translatable MT mRNA increase prior to increases in hepatic Zn-MT levels (67). These findings support a role for MTs in controlling zinc concentrations in cells under normal dietary conditions. Furthermore, since MT gene expression is decreased when the dietary zinc intake is restricted, the concomitant decrease in cellular MT could relate to observed signs of zinc deficiency.

MT gene expression is elevated in several tissues during fetal development (81–84) suggesting a role in controlling metal ion concentrations important to growth and development. This idea is supported by the finding that growth-arrested human fibroblasts have lower levels of MT mRNA than do actively proliferating cells (85). It has been shown, however, that cell lines that have lost their ability to make MTs are fully viable and grow normally, arguing against an essential role for MTs in growth (70, 72). Because many zinc- and copper-requiring enzymes are involved in growth this also argues against any essential role of MT in enzyme activation. Damage caused by specific toxins may lead to MT synthesis in new fetal-like cells in adult organs.

When mutant yeast cell lines are made with deletion of their MT-like protein (CUP1) coding sequences but with retention of the CUP1 promoter sequences, higher than normal rates of gene transcription initiated by the CUP1 promoter are exhibited, even in low-copper media (86). But, when mammalian MT coding sequences are reinserted and their expression

is also controlled by the CUP1 promoter, normal (reduced) transcriptional rates are restored indicating that MT chelates copper that would otherwise have activated transcription (87). This argues in favor of MT functioning to maintain low levels of free copper in the cell (3).

The induction of MTs by glucocorticoids, epinephrine, or inflammatory agents results in reduced plasma Zn concentration with concomitant increases in hepatic zinc (reviewed in (2)). At 10 hr after induction by various agents, hepatic MT levels are inversely related to this concentration (41). Kinetic studies using MT induction by cAMP have shown that much of the increased zinc taken up by the liver is bound to MT (43). Furthermore, MT-bound zinc after cAMP treatment exhibits rapid exchange with ^{65}Zn in the plasma compartment. Without MT induction little rapidly exchanged ^{65}Zn binds to MT. Therefore, in hormonal regulation of synthesis, mobilization of zinc into MT is an effect of MT induction rather than a cause of induction. The physiological significance of this mobilization is unclear but it may play a role in cellular defense mechanisms or in requisite reduction in plasma zinc.

The fact that MTs are induced by various types of stress and by hormones and other agents elevated by stress or inflamation (i.e., glucocorticoids, interleukins, interferons, endotoxins) further suggests that MT is involved in cellular defense mechanisms (reviewed in (2)). Recently, MT has been shown to be an efficient scavenger of free hydroxyl (• OH) ions *in vitro* (88). Evidence for this potential function centers on the markedly greater reactivity of MT with • OH radicals compared to superoxide radicals. It is envisioned that following reaction with the • OH radical, metal loss from MT and thiolate oxidation occurs. Regeneration of the metalloprotein could involve reduction with GSH and an appropriate divalent cation. If these reactions occur *in vivo,* scavenging of free radicals released during the acute phase response could protect tissues from damage. Prevention of lipid peroxidation in hepatocytes in culture by zinc supplementation of culture medium has been correlated to MT induction (89). Free radicals are also responsible for tissue damage resulting from ionizing radiation. It has been observed that cells which overproduce MTs are more resistant to X-ray damage than normal cells (90) and that X-ray treatment induces hepatic and renal MTs in intact rats (91). Also, uv light has been reported to induce MTs (92). MT may function, then, to protect cells in a number of situations by reducing damage from free radicals. Alternatively, the protein may serve as a source of zinc for enzymes that repair DNA or other tissue damage.

Medical Implications. In light of the ability of MTs to bind heavy metals and possibly regulate their metabolism, as well as function as free-radical scavengers, these unique proteins may play a role in either the prevention, treatment, or etiology of certain diseases.

It seems certain that MTs protect cells on a practical scale from several types of metal toxicities. For example, under conditions of hepatic copper accumulation such as Wilson's disease and in fetal liver, MTs appear to sequester large amounts of copper in a relatively nontoxic form (56–59). Negative copper balance in Wilson's disease patients is produced by oral zinc therapy which may induce intestinal MT and promote copper retention in this tissue (93). This may be a beneficial treatment for patients once copper accumulation has been stabilized by chelation therapy. Also, as previously mentioned, pretreatment of animals with zinc to stimulate MT production may afford protection against lethal cadmium exposure (1). Increased urinary MT observed in factory workers exposed to large amounts of cadmium may be a manifestation of that protective response (94).

Copper accumulation associated with Menkes' disease and primary biliary cirrhosis (PBC) may be related to MT. In Menkes' disease this may be due to a defect in the regulation of Cu-MT turnover such that MT-bound copper becomes unavailable to important copper-requiring enzymes leading to symptoms of copper deficiency (66, 95). In PBC, induction of hepatic MT may contribute to the accumulation of high concentrations of copper in this organ (96). MTs can also be problematic in the treatment of certain diseases. Evidence suggests that MTs can be induced by and bind metals like gold and platinum contained in potentially therapeutic

drugs thereby reducing the effectiveness of these compounds (97, 98).

The finding that MTs are induced by hormones such as interleukin and glucocorticoids, which are elevated during infection, suggests that MTs may somehow be involved in defense mechanisms. Although speculative at this time, such involvement could be related to the potential function of MTs as a scavenger of damaging free radicals produced either by the body's protective response to infection or from exposure to X-ray or uv radiation. Free-radical scavenging could also be preventative in cancer initiation.

Involvement of MTs in host defense may also be related to the finding that hormonal induction of hepatic MTs results in an alteration in zinc distribution between tissues and within cells. Plasma zinc levels are reduced while Zn and Zn-MT levels within hepatocytes are greatly increased (41). Possibly other tissues and cells such as macrophages are affected as well. Macrophages, which are among the first cells to encounter gram-negative bacteria, do synthesize MT under conditions of stress and may accumulate zinc (46, 99). Such redistribution of zinc may be involved in some way in increasing resistance to infection. For example, macrophages are more resistant to endotoxin when MT has been induced (99). Also, as mentioned above, natural killer activity of lymphocytes is stimulated *in vitro* by treatments that stimulate MTs (45).

Finally, the immunochemical detection of low concentrations of MT in plasma coupled with the positive correlations between zinc status and plasma Zn-MT levels suggests that plasma MT levels could be diagnostic for zinc status (100).

Summary. Early research on metallothionein centered on aspects related to a detoxification role. As our understanding of the complex endocrine control that regulates metallothionein gene expression increases, a wider appreciation of a functional role(s) is emerging. Medical implications of control of metallothionein turnover include diagnosis of specific diseases and regulation of its expression as a host defense component.

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1. Kagi JHR, Nordberg M. (Eds.). Metallothionein. Basel: Birkhäuser-Verlag, 1979.
2. Cousins RJ. Absorption, transport, and hepatic metabolism of copper and zinc: Special reference to metallothionein and ceruloplasmin. Physiol Rev **65**(2):238–309, 1985.
3. Hamer DH. Metallothionein. Ann Rev Biochem **55:** 913–951, 1986.
4. Kagi JHR, Kokima Y. (Eds.). Metallothionein-2. Basel: Birkhäuser-Verlag, in press.
5. Danielson KG, Ohi S, Huang PC. Immunochemical detection of metallothionein in specific epithelial cells of rat organs. Proc Natl Acad Sci USA **79:**2301–2304, 1982.
6. Banerjee D, Onosaka S, Cherian MG. Immunohistochemical localization of metallothionein in cell nucleus and cytoplasm of rat liver and kidney. Toxicol **24:**95–105, 1982.
7. Cherian MG, Nordberg M. Cellular adaption in metal toxicology and metallothionein. Toxicol **28:**1–15, 1983.
8. Garvey JS, Chang CC. Detection of circulating metallothionein in rats injected with zinc or cadmium. Science **211:**805–807, 1981.
9. Bremner I, Mehra RK, Morrison JN, Wood AM. Effects of dietary copper supplementation of rats on the occurrence of metallothionein-I in liver and its secretion into blood, bile and urine. Biochem J **235:** 735–739, 1986.
10. Klauser S, Kagi JHR, Wilson KJ. Characterization of isoprotein patterns in tissue extracts and isolated samples of metallothioneins by reverse-phase high-pressure liquid chromatography. Biochem J **209:**71–80, 1983.
11. Nielson KB, Winge DR. Preferential binding of copper to the β domain of metallothionein. J Biol Chem **259:**4941–4946, 1984.
12. Hartmann H, Weser U. Copper-thionein from fetal bovine liver. Biochim Biophys Acta **491:**211–222, 1977.
13. Ryden L, Deutsch HF. Preparation and properties of the major copper-binding component in human fetal liver. Its identification as metallothionein. J Biol Chem **253:**519–524, 1978.
14. Minkel DT, Poulsen K, Wielgus S, Shaw CF III, Petering DH. On the sensitivity of metallothioneins to oxidation during isolation. Biochem J **191:**475–485, 1980.
15. Eaton DL, Toal BF. Evaluation of the Cd/hemoglobin affinity assay for the rapid determination of metallothionein in biological tissues. Toxicol Appl Pharmacol **66:**134–142, 1982.
16. Scheuhammer AM, Cherian MG. Quantification of metallothioneins by a silver-saturation method. Toxicol Appl Pharmacol **82:**417–425, 1986.
17. Zelazowski AJ, Piotrowski JK. A modified procedure for determination of metallothionein-like proteins in animal tissues. Acta Biochim Pol **24:**97–103, 1977.
18. Garvey JS, Vander Mallie RJ, Chang CC. (Eds.). Radioimmunoassay of metallothioneins. Met Enzym **84:**121–139, 1982.
19. Mehra FK, Bremner I. Development of a radioim-

munoassay for rat liver metallothionein-1 and its application to the analysis of rat plasma and kidneys. Biochem J **213**:459–465, 1983.

20. Thomas DG, Linton HJ, Garvey JS. Fluorometric ELISA for the detection and quantitation of metallothionein. J Immunol Methods **89**:239–247, 1986.
21. Dieter HH, Muller L, Abel J, Summer KH. Determination of Cd-thionein in biological materials: Comparative standard recovery by five current methods using protein nitrogen for standard calibration. Toxicol Appl Pharmacol **85**:380–388, 1986.
22. Kagi JHR, Himmelhoch SR, Whanger PD, Bethune JL, Vallee BL. Equine hepatic and renal metallothioneins. Purification, molecular weight, amino acid composition, and metal content. J Biol Chem **249**: 3537–3542, 1974.
23. Otvos JD, Armitage IM. Structure of the metal clusters in rabbit liver metallothionein. Proc Natl Acad Sci USA **77**:7094–7098, 1980.
24. Winge DR, Miklossy KA. Domain nature of metallothionein. J Biol Chem **257**:3471–3476, 1982.
25. Nielson KB, Atkin CL, Winge DR. Distinct metal-binding configurations in metallothionein. J Biol Chem **260**:5342–5350, 1985.
26. Furey WF, Robbins AH, Clancy LL, Winge DR, Wang BC, Stout CD. Crystal structure of Cd, Zn metallothionein. Science **231**:704–710, 1986.
27. Winge DR, Nielson KB, Zeikus RD, Gray WR. Structural characterization of the isoforms of neonatal and adult rat liver metallothionein. J Biol Chem **259**:11419–11425, 1984.
28. Nielson KB, Winge DR. Independence of the domains of metallothionein in metal binding. J Biol Chem **260**(15):8698–8701, 1985.
29. Nettesheim DG, Engeseth HR, Otvos JD. Products of metal exchange reactions of metallothionein. Biochemistry **24**:6744–6751, 1985.
30. Shaikii, ZA. The low molecular weight cadmium, mercury and zinc binding proteins (metallothionein): Biosynthesis, metabolism and possible role in metal toxicity. In: Kagi JHR, Nordberg M, Eds. Metallothionein. Basel: Birkhäuser, pp331–336, 1979.
31. Webb M. Binding of cadmium ions by rat liver and kidney. Biochem Pharmacol **21**:2751–2765, 1972.
32. Squibb KS, Cousins RJ. Control of cadmium binding protein synthesis in rat liver. Environ Physiol Biochem **4**:24–30, 1974.
33. Squibb KS, Cousins RJ, Feldman SI. Control of zinc-thionein synthesis in rat liver. Biochem J **164**:223–228, 1977.
34. Richards MP, Cousins RJ. Influence of parenteral zinc and actinomycin D on tissue zinc uptake and the synthesis of a zinc-binding protein. Bioinorg Chem **4**:215–224, 1975.
35. Durnam DM, Palmiter RD. Transcriptional regulation of the mouse metallothionein-I gene by heavy metals. J Biol Chem **256**(11):5712–5716, 1981.
36. Blalock TL, Dunn MA, Cousins RJ. Sensitivity of native metallothionein promoters to dietary copper and zinc. Fed Proc **46**:884, 1987. (Abstract). In press.
37. Durnam DM, Palmiter RD. Induction of metallothionein-I mRNA in cultured cells by heavy metals and iodoacetate: Evidence for gratuitous inducers. Mol Cell Biol **4**(3):484–491, 1984.
38. Searle PF, Davison BL, Stuart GW, Wilkie TM, Norstedt G, Palmiter RD. Regulation, linkage, and sequence of mouse metallothionein I and II genes. Mol Cell Biol **4**(7):1221–1230, 1984.
39. O'Halloran T, Walsh C. Metalloregulatory DNA-binding protein encoded by the merR gene: Isolation and characterization. Science **235**:211–214.
40. Hager LG, Palmiter RD. Transcriptional regulation of mouse liver metallothionein-I gene by glucocorticoids. Nature (London) **291**:340–342, 1981.
41. Cousins RJ, Dunn MA, Leinart AS, Yedinak KC, DiSilvestro RA. Coordinate regulation of zinc metabolism and metallothionein gene expression in rats. Amer J Physiol **251**:E688–E694, 1986.
42. Mayo KE, Palmiter RD. Glucocorticoid regulation of the mouse metallothionein I gene is selectively lost following amplification of the gene. J Biol Chem **257**(6):3061–3067, 1982.
43. Dunn MA, Cousins RJ. Regulation of metallothionein gene transcription and kinetics of zinc metabolism by dibutyryl cAMP. Fed Proc **46**:598, 1987. (Abstract). In press.
44. Friedman RL, Manly SP, McMahon M, Kerr IM, Stark GR. Transcriptional and post-transcriptional regulation of interferon-induced gene expression in human cells. Cell **38**:745–755, 1984.
45. Cunningham-Rundles S. Nutritional factors in immune response. In: Malnutrition: Determinants and Consequences. New York: Liss, pp233–244, 1984.
46. Patierno SR, Costa M, Lewis VM, Peavy DL. Inhibition of LPS toxicity for macrophages by metallothionein-inducing agents. J Immunol **130**:1924–1929, 1983.
47. Sobocinski PZ, Canterbury WJ Jr. Hepatic metallothionein induction in inflammation. Ann NY Acad Sci **210**:354–367, 1982.
48. DiSilvestro RA, Cousins RJ. Mediation of endotoxin-induced changes in zinc metabolism in rats. Amer J Physiol **247**:E436–E441, 1984.
49. Swerdel MR, Cousins RJ. Changes in rat liver metallothionein and metallothionein mRNA induced by isopropanol. Proc Soc Exp Biol Med **175**:522–529, 1984.
50. Durnam DM, Hoffman JS, Quaife CJ, Benditt EP, Chen HY, Brinster RL, Palmiter RD. Induction of mouse metallothionein-I mRNA by bacterial endotoxin is independent of metals and glucocorticoid hormones. Proc Natl Acad Sci USA **81**:1053–1056, 1984.
51. DiSilvestro RA, Cousins RJ. Glucocorticoid independent mediation of interleukin-1 induced changes in serum zinc and liver metallothionein levels. Life Sci **35**:2113–2118, 1984.

52. Richards MP, Cousins RJ. Metallothionein and its relationship to the metabolism of dietary zinc in rats. J Nutr **106**:1591–1599, 1976.
53. Hidalgo J, Armario A, Flos R, Dingman A, Garvey JS. The influence of restraint stress in rats on metallothionein production and corticosterone and glucagon secretion. Life Sci **39**:611–616, 1986.
54. Bremner I, Hoekstra WG, Davies NT, Young BW. Effect of zinc status of rats on the synthesis and degradation of copper induced metallothionein. Biochem J **174**:883–892, 1978.
55. Feldman SL, Squibb KS, Cousins RJ. Degradation of cadmium thionein in rat liver and kidney. J Toxicol Environ Health **4**:805–813, 1978.
56. Feldman SL, Cousins RJ. Degradation of hepatic zinc-thionein following parenteral zinc administration. Biochem J **160**:583–588, 1976.
57. Feldman SI, Failla ML, Cousins RJ. Degradation of liver metallothioneins in vitro. Biochim Biophys Acta **544**:638–646, 1978.
58. Held DD, Hoekstra WG. In vitro degradation of metallothionein by rat liver lysosomes. Fed Proc **38**: 2674, 1979. (Abstract)
59. Bremner I, Mehra RK. Metallothionein: Some aspects of its structure and function with special regard to its involvement in copper and zinc metabolism. Chem Sci **21**:117–121, 1983.
60. Ridlington JW, Winge DR, Fowler BA. Long-term turnover of cadmium metallothionein in liver and kidney following a single low dose of cadmium in rats. Biochim Biophys Acta **673**:177–183, 1981.
61. Whanger PD, Ridlington JW. Role of metallothionein in zinc metabolism. In: Foulkes EC, Ed. Biological Roles of Metallothionein. New York: Elsevier, pp263–279, 1982.
62. Johnson GF, Morell AG, Stockert RJ, Sternlieb I. Hepatic lysosomal copper protein in dogs with an inherited copper toxicosis. Hepatology **1**:243–248, 1981.
63. Mehra RK, Bremner I. Species difference in the occurrence of copper-metallothionein in the particulate fractions of the liver of copper-loaded animals. Biochem J **219**:539–545, 1984.
64. Riordan JR, Richards V. Human fetal liver contains both zinc- and copper-rich forms of metallothionein. J Biol Chem **255**:5380–5383, 1980.
65. Sternlieb I. Copper and the liver. Gastroenterology **78**:1615–1628, 1980.
66. Prins HW, Van den Hamer CJA. Comparative studies of copper metabolism in liver and kidney of normal and mutated brindled mice—with special emphasis on metallothionein. Comp Biochem Physiol C **70**:255–260, 1981.
67. Seagrave JC, Hanners JL, Taylor W, O'Brien HA. Transfer of copper from metallothionein to nonmetallothionein proteins in cultured cells. Biol Trace Elem Res **10**:163–173, 1986.
68. McCormick CC, Menard MP, Cousins RJ. Induction of hepatic metallothionein by feeding zinc to rats of depleted zinc status. Amer J Physiol **240**:E414–E421, 1981.
69. Pattison SE, Cousins RJ. Zinc uptake and metabolism by hepatocytes. Fed Proc **45**:2805–2809, 1986.
70. Compere SJ, Palmiter RD. DNA methylation controls the inducibility of mouse metallothionein-I gene in lymphoid cells. Cell **25**:233–240, 1981.
71. Karin M, Cathala G, Nguyenhu MC. Expression and regulation of a human metallothionein gene carried on an autonomously replicating shuttle vector. Proc Natl Acad Sci USA **80**:4040–4044, 1983.
72. Crawford BD, Enger MD, Griffith BB, Griffith JK, Hanners JL, Longmire JL, Munk AC, Stallings RL, Tesmer JG, Walkers RA, Hildebrand CE. Coordinate amplification of metallothionein I and II genes in cadmium-resistant Chinese hampster cells: Implications for mechanisms regulating metallothionein gene expression. Mol Cell Biol **5**:320–329, 1985.
73. Pattison SE, Cousins RJ. Kinetics of zinc uptake and exchange by primary cultures of rat hepatocytes. Amer J Physiol **250**(13):E677–E685, 1986.
74. Williams RJP. Zinc: What is its role in biology? Endeavour **8**(2):65–70, 1984.
75. Grider A Jr, Erway LC. Intestinal metallothionein in lethal-milk mice with systemic zinc deficiency. Biochem Genet **24**:635–642, 1986.
76. Brewer GJ, Hill GM, Prasad AS, Cossack ZT, Rabbani P. Oral zinc therapy for Wilson's disease. Ann Intern Med **99**:314–320, 1983.
77. Udom AO, Brady FO. Reactivation in vitro of zinc-requiring apo-enzymes by rat liver zinc-thionein. Biochem J **187**:329–335, 1980.
78. Scheckinger T, Hartmann HJ, Weser U. Copper transport from Cu(I)-thionein into apo-caeruloplasmin mediated by activated leucocytes. Biochem J **240**:281–283, 1986.
79. Li TY, Kramer AJ, Shaw CF, Petering DH. Ligand substitution reactions of metallothioneins with EDTA and apo-carbonic anhydrase. Proc Natl Acad Sci USA **77**:6334–6338, 1980.
80. Lerch K. Copper metallothionein, a copper-binding protein from neurospora crassa. Nature (London) **284**:368–370, 1980.
81. Ouellette AJ. Metallothionein mRNA expression in fetal mouse organs. Dev Biol **92**:240–246, 1982.
82. Anderson RD, Piletz JE, Birren BW, Herschman HR. Levels of metallothionein messenger RNA in foetal, neonatal and maternal rat liver. Eur J Biochem **131**: 497–500, 1983.
83. Andrews GK, Adamson ED, Gedamu L. The ontogeny of expression of murine metallothionein: Comparison with the α-fetoprotein gene. Dev Biol **103**:294–303, 1984.
84. Mercer JFB, Grimes A. Variation in the amounts of hepatic copper, zinc and metallothionein mRNA during development in the rat. Biochem J **238**:23–27, 1986.
85. Angel P, Poting A, Mallick U, Rahmsdor HJ, Schorpp M, Herrlich P. Induction of metallothionein

and other messenger-RNA species by concinogeus and tumor promoter in primary human skin fibroblasts. Mol Cell B **6**(5):1760–1766, 1986.

86. Hamer DH, Thiele DJ, Lemontt JE. Function and autoregulation of yeast copper thionein. Science **228**: 685–690, 1985.
87. Thiele D, Walling MJ, Hamer D. Mammalian metallothionein is functional in yeast. Science **231**:854–856, 1986.
88. Thornalley PJ, Vasak M. Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. Biochim Biophys Acta **827**:36–44, 1985.
89. Cousins RJ, Coppen DE. Regulation of liver zinc metabolism and metallothionein by cAMP, glucagon and glucocorticoids and suppression of free radicals by zinc. In: Kagi JHR, Ed. Metallothionein. Basel: Birkhäuser-Verlag, 1987. In press.
90. Bakka A, Webb M. Metabolism of zinc and copper in the neonate: Changes in the concentrations and contents of thionein-bound Zn and Cu with age in the livers of the newborn of various mammalian species. Biochem Pharmacol **30**:721–725, 1981.
91. Shiraishi N, Yamamoto H, Takeda Y, Kondoh S, Hayashi H, Hashimoto K, Aono K. Increased metallothionein content in rat liver and kidney following X-irradiation. Toxicol Appl Pharmacol **85**:128–134, 1986.
92. Herrlich P, Rahmsdor HJ, Angel P, Luckehuh C, Jonat C, Eades AM, Karin M, Cato A, Ponta H. Signals and sequences involved in the UV and TPA dependent induction of genes. J Cell Biol **(S10C)**: 108, 1986. (Abstract)
93. Brewer GJ, Hill GM, Prasad AS, Cossack ZT, Rabbani P. Oral zinc therapy for Wilson's disease. Ann Intern Med **99**:314–320, 1983.
94. Chang CC, Lauwerys R, Bernard A, Roels H, Buchet JP, Garvey JS. Metallothionein in cadmium-exposed workers. Environ Res **23**:422–428, 1980.
95. Riordan JR, Jolicoeur-Paquet L. Metallothionein accumulation may account for intracellular copper retention in Menkes' disease. J Biol Chem **257**:4639–4645, 1982.
96. Salaspuro MP, Pikkarainen P, Sipponen P, Vuori E, Miettinen TA. Hepatic copper in primary biliary cirrhosis: Biliary excretion and response to penicillamine treatment. Gut **22**:901–906, 1981.
97. Butt TR, Sternberg EJ, Mirabelli CK, Crooke ST. Regulation of metallothionein gene expression in mammalian cells by gold compounds. Mol Pharmacol **29**:204–210, 1985.
98. Bakka A, Endresen L, Johnsen ABS, Edminson PD, Rugstad HE. Resistance against cis-dichlorodiammineplatinum in cultured cells with a high content of metallothionein. Toxicol Appl Pharmacol **61**:215–226, 1981.
99. Patierno SR, Peavy DL. Induction of metallothionein in macrophages: A molecular mechanism for protection against LPS-mediated autolysis. Immunopharmacol Endotoxicosis 39–56, 1984.
100. Sato M, Mehra RK, Bremner I. Measurement of plasma metallothionein-I in the assessment of the zinc status of zinc-deficient and stressed rats. J Nutr **114**:1683–1689, 1984.