Biometals (2010) 23:897–926
DOI 10.1007/s10534-010-9351-z

Role of metallothionein in cadmium traffic and toxicity in kidneys and other mammalian organs

Ivan Sabolić · Davorka Breljak · Mario Škarica · Carol M. Herak-Kramberger

Received: 30 November 2009 / Accepted: 28 May 2010 / Published online: 15 June 2010

Abstract Metallothioneins are cysteine-rich, small metal-binding proteins present in various mammalian tissues. Of the four common metallothioneins, MT-1 and MT-2 (MTs) are expressed in most tissues, MT-3 is predominantly present in brain, whereas MT-4 is restricted to the squamous epithelia. The expression of MT-1 and MT-2 in some organs exhibits sex, age, and strain differences, and inducibility with a variety of stimuli. In adult mammals, MTs have been localized largely in the cell cytoplasm, but also in lysosomes, mitochondria and nuclei. The major physiological functions of MTs include homeostasis of essential metals Zn and Cu, protection against cytotoxicity of Cd and other toxic metals, and scavenging free radicals generated in oxidative stress. The role of MTs in Cd-induced acute and chronic toxicity, particularly in liver and kidneys, is reviewed in more details. In acute toxicity, liver is the primary target, whereas in chronic toxicity, kidneys are major targets of Cd. The intracellular MTs bind Cd ions and form CdMT. In chronic intoxication, Cd stimulates de novo synthesis of MTs; it is assumed that toxicity in the cells starts when loading with Cd ions exceeds the buffering capacity of intracellular MTs. CdMT, released from the Cd-injured organs, or when applied parenterally for experimental purposes, reaches the kidneys via circulation, where it is filtered, endocytosed in the proximal tubule cells, and degraded in lysosomes. Liberated Cd can immediately affect the cell structures and functions. The resulting proteinuria and CdMT in the urine can be used as biomarkers of tubular injury.

Keywords Antioxidants · Heavy metals · Hepatotoxicity · Nephrotoxicity · Oxidative stress · Reactive oxygen species · Urine metallothionein

Abbreviations

Apo-MT	Metallothionein protein not complexed with metal
BBM	Brush-border membrane
BLM	Basolateral membrane
DMT-1	Divalent metal transporter 1
GIF	Growth inhibitory factor (MT-3)
GSH	Reduced glutathione
GSSG	Oxidized glutathione
MT	Metallothionein
MTF-1	Metalloregulatory transcription factor 1
MT-null mice	MT-1 and MT-2 knock out mice
Oat	Organic anion transporter

I. Sabolić (✉) · D. Breljak · M. Škarica · C. M. Herak-Kramberger
Unit of Molecular Toxicology, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10001 Zagreb, Croatia
e-mail: sabolic@imi.hr

Present Address:
M. Škarica
School of Medicine, Department of Microbiology and Immunology, Autoimmunity Center, Temple University, Philadelphia, PA 19140, USA

PT	Proximal tubules
RE	Response element
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
ZnMT	CdMT or CuMT, metallothionein complexed with Zn, Cd or Cu, respectively

Molecular characteristics of mammalian metallothioneins

Metallothioneins (MTs) are metal-binding proteins found in various mammalian and non-mammalian tissues. The first mammalian MT was isolated from the equine kidney cortex (Kägi and Vallee 1960). Later studies revealed in mammalian organs a whole family of evolutionary conserved, heat-stable and low MW (~7 kDa) single chain polypeptides, with 61–68 amino acids that contain 20 cysteines in a reduced form, but no aromatic amino acid and histidine residues. The metal-free protein part of MTs (thionein, apo-MT) is characterized by six C-X-C repeats (where C is cysteine and X can be any other amino acid; Fig. 1, red-labeled sequences) and one distinct sequence pattern K-X(1,2)-C-C-X-C-C-P-X(2)-C (Fig. 1, blue-labeled sequence). These specific sequences are structurally arranged in two unique metal domains, e.g., α and β clusters, each having different metal binding capacity (Winge and Miklossy 1982). Due to numerous cysteines with thiol (SH) groups, MTs exhibit high-affinity/high-capacity binding properties for various reactive metal ions, such as Zn, Cd, Hg, Cu, Pb, Ni, Co, Fe, Ag, and Au; one molecule of apo-MT can bind 7–9 Zn or Cd ions (or any combination of these two), up to 12 Cu ions, and up to 18 Hg ions (Palumaa et al. 2002, 2003; reviewed in Stillman 1995). The reactive thiol groups promote spontaneous oligomerization of the thionein molecules, and such aggregates from the rabbit liver and horse kidney can bind much more than usual 7 g atom Cd/mol apo-MT (Zangger et al. 2001; Wilhelmsen et al. 2002). The binding affinity of MTs for metal ions is metal-dependent, but several in vitro studies, using rat liver common MTs, showed a slightly different relative order of affinities for metals; in the study by Waalkes et al. (1984), the order was Cd > Pb > Cu > Hg > Zn > Ag > Ni > Co, in the study by Nielson et al. (1985), the order was Hg > Cu > Cd > Zn > Ni = Co, whereas Hamer (1986) reported the order: Hg > Ag > > Cu > Cd > Zn. Furthermore, the estimated stability constant of MTs for Zn (10^{10}–10^{11} M^{-1}) and for Cd (10^{13}–10^{14} M^{-1}) show that CdMT has 100–1000-fold higher stability than ZnMT (reviewed in Romero-Isart and Vašak 2002). These data indicate that higher affinity metals, such as Cd, Hg, or Cu, can displace Zn and other lower affinity metals from the metal-MT

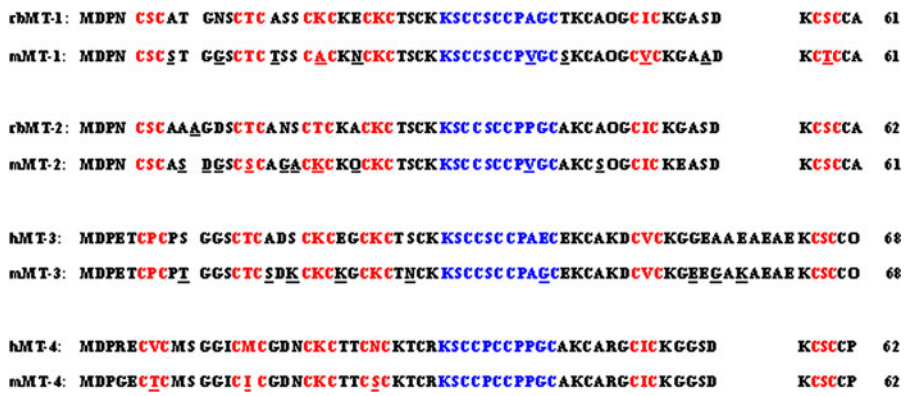

Fig. 1 Alignment of amino acid sequences in four common MTs of mammalian cells; Species differences. *rb* rabbit, *m* mouse, *h* human. Species differences of specific amino acids are *underlined*. The number of amino acid residues in the protein is indicated at the end of each sequence. The *red*-labeled sequences denote C-X-C repeats (where C is cysteine and X can be any other amino acid), whereas the *blue*-labeled sequence denotes a characteristic pattern K-X(1,2)-C-C-X-C-C-P-X(2)-C common to all mammalian MTs. The data have been collected from Hunziker et al. (1995), Palmiter et al. (1992), and Quaife et al. (1994)

complex; such pattern was indeed experimentally proven for Cd action upon horse ZnMT in vitro (Kägi and Vallee 1960), and for Cd and Hg actions upon ZnMT in the rat liver in vivo (Day et al. 1984). Furthermore, the initial in vitro study by Kägi and Vallee (1960) showed that Zn and Cd can spontaneously dissociate from the metal–MT complex at low pH, and that Cd, due to higher affinity for apo-MT, dissociates slower than Zn. Later, more detailed in vitro and in vivo studies showed that: (a) acidic medium, such as present in lysosomes (pH 5.5), promotes dissociation of the metal from the metal–MT complex, (b) high affinity metal–MT complexes, particularly those fully saturated with metals, exhibit much higher resistance to degradation by lysosomal proteases than the less saturated complexes or the apo-MTs alone; CdMT has 2–3 times longer half life than ZnMT, and (c) degradation of apo-MT in the acidic (lysosomal) medium is ~390-fold faster than in the neutral (cytoplasmic) medium (Feldman et al. 1978; Choudhuri et al. 1992; Kershaw and Klaassen 1992; McKim et al. 1992; Klaassen et al. 1994;). However, the measurements in various tumor cells and established cell lines (Pattanaik et al. 1994; Petering et al. 2006), and in fresh tissues from various rat organs (Yang et al. 2001), revealed that only a part of MTs in the cells/tissues is complexed with Zn, whereas a significant proportion of the total MT (up to 90% in some cell lines and tumor cells, and up to 54% in the rat tissues) exists as the metal-free form. These studies also showed that, unlike the situation in acidic lysosomes, in cytoplasmic conditions (pH 7) apo-MT is more stable than the metal-saturated MT (Yang et al. 2001; reviewed in Petering et al. 2006).

Metallothionein genes and their products

The mammalian genome contains multiple MT genes, which encode at least four thioneins. MTs in rodents are controlled by four genes located on chromosome 8 (MT-I, MT-II, MT-III and MT-IV, all four functional, which code for apo-proteins MT-1, MT-2, MT-3 and MT-4, respectively), whereas human MTs are controlled by 17 genes located on chromosome 16, of which 10 are functional (reviewed in Miles et al. 2000). As shown by a few examples in Fig. 1, in different species the amino acid sequence of the common four MTs exhibits significant polymorphism. In addition, each form exhibits multiple isoforms which can be located in the same or different cells/tissues/organs, but their precise physiological roles and functional characteristics in different localizations are largely unclear.

MT-1 and MT-2 represent the most prevalent thionein isoforms expressed in variable abundance in all mammalian tissues. These two isoforms have been most extensively studied, particularly in relation to their roles in homeostasis of essential metals (Zn, Cu) and Cd toxicity in various organs, and will be the major focus of interest in the following pages. The knock-out (KO) mice for MT-1 or MT-2 (mice with inactivated genes and nominal absence of the respective proteins), or for both (MT-null mice) genes, as well as transgenic (TG) mice (mice with multiple identical MT genes and overproduction of the respective proteins) for one or both MT genes have been generated, and extensively exploited in studying roles of MTs in various organs in intact and Cd-intoxicated animals (Masters et al. 1994; Iszard et al. 1995; Dalton et al. 1996b; Liu et al. 1996a; reviewed in: Klassen et al. 1999, 2009; Andrews 2000; Davis and Cousins 2000; Nordberg and Nordberg 2000). In rabbits, rats and mice in vivo, and in the respective cultured cells from animal or human origin, Zn, Cd, and oxidative stress were shown to be particularly strong inducers of both genes in liver and kidneys, as manifested by strong enhancement of MT-1/MT-2 content in both organs (Waalkes and Klaassen 1985; Klaassen and Lehman-McKeeman 1989; reviewed in: Hamer 1986; Klaassen and Liu 1997; Nordberg 1998; Palmiter 1998; Klaassen et al. 1999, 2009; Davis and Cousins 2000). At least in the rat liver, the abundance of MT-2 was found to be higher than that of MT-1, possibly due to higher stability of ZnMT-2 (apparent half life 21.9 h) compared to ZnMT-1 (apparent half life 12.2 h). The expression of these isoforms and their mRNAs was somehow differently affected in Cd-, Zn- and dexamethasone-treated rats; Cd and Zn induced both isoforms to a similar extent, whereas dexamethasone induced predominantly MT-2 (Klaassen and Lehman-McKeeman 1989). Some additional discrepancies were found in Cd- and Cu-treated rats for the expression levels of MT-1 and MT-2 mRNAs and proteins in the liver and kidneys (Vasconcetos et al. 2002). However, many studies described in the literature have been performed without discriminating

effects on MT-1 and MT-2, and have been collectively presented as "on MT". In addition, in most immunochemical studies the anti-MT antibodies did not discriminate between these two isoforms. Therefore, in further discussion the term "metallothionein (MT)" will be used as a term common to both MTs (MT-1 and MT-2).

The expression of MT-3 was initially detected in specific structures of the mouse and human brain, such as the Zn-rich neurons and astrocytes in the cortex, hippocampus, and amigdala, and found to be a growth inhibitory factor (GIF) for cultured neurons (reviewed in: Ebadi et al. 1995; Aschner et al. 1997). The expression of brain MT-3 in rodents in vivo was either weakly downregulated (Zheng et al. 1995) or unresponsive (Palmiter et al. 1992; reviewed in: Ebadi et al. 1995; Aschner et al. 1997; Aschner and West 2005) to the well known inducers of MT-1/MT-2, such as Zn, Cd, dexamethasone, and bacterial endotoxin. The roles of MT-3 as the intracellular regulator of Zn and Cu, scavenger of reactive oxygen species (ROS), GIF, and neuroprotector in brain physiology, pathology and toxicity, have been described in several reviews (Ebadi et al. 1995; Aschner et al. 1997; Mendez-Armenta and Rios 2007). Other studies have reported that the Cd-insensitive MT-3 mRNA is also present in variable amounts in male and female urogenital tracts, and in a few other peripheral organs of rats, mice and humans (Moffatt and Seguin 1998; Hozumi et al. 2008). In the human kidney, MT-3 was detected in glomerular, proximal and distal tubule cells, where it may contribute to transport functions and mechanisms of cell death (Hoey et al. 1997; Garrett et al. 1999; Kim et al. 2002; Somji et al. 2004). A study in cultured human proximal tubule (PT) cells showed that MT-3 can be induced weakly and transiently by Zn or Cd (Garrett et al. 2002). On the other side, the expression of MT-4 mRNA in mice and humans seems to be exclusively present in stratified squamous epithelia (oral cavity, upper gastrointestinal tract, skin, footpads), where it may serve homeostasis of Zn and Cu during cell differentiation (Quaife et al. 1994), but its behavior in metal-induced toxicity is not known.

Regulation of metallothionein gene activity

MT mRNA and/or protein are highly inducible. Besides essential (Zn, Cu) and toxic metals, a variety of other compounds and factors, such as hormones and secondary messengers, growth factors, inflammatory and cytotoxic agents, tumor promoters and oncogenes, antibiotics, vitamins and food components, as well as various stress-related conditions, upregulate the MT production, largely via generating oxidative stress and free radicals (ROS and reactive nitrogen species (RNS)) in the affected cells (Oh et al. 1978; reviewed in Nordberg 1998; Nordberg and Nordberg 2009).

The induction of MT primarily results from an enhanced transcription of structural genes following interaction of specific stimuli with response elements (RE) in the gene promoter (reviewed in: Samson and Gedamu 1998; Andrews 2000; Davis and Cousins 2000; Vašak and Hasler 2000; Haq et al. 2003; Petering et al. 2009). The schematic representation of REs and their activating ligands in the MT promoter from the mouse liver is shown in Fig. 2. One or more REs exist for glucocorticoids (GRE), metals (MRE), cytokines and other inflammatory molecules, and for hydrogen peroxide (H_2O_2) and other free radicals (antioxidant RE, ARE). A detailed description of the significance and regulatory functions of various REs in the mammalian (mouse) MT promoter was reviewed elsewhere (Samson and Gedamu 1998; Andrews 2000; Davis and Cousins 2000; Vašak and Hasler 2000; Haq et al. 2003). The in vivo and in vitro studies have shown that the metalloregulatory transcription factor MTF-1 is the major player in regulating MT gene expression in physiological conditions and in response to metal ions. The data in MTF-1 KO mice showed that this Zn-finger protein plays a critical role in embryonic liver formation, hematopoiesis, and in oxidative stress due to Cd toxicity (Gunes et al. 1998; Wang et al. 2004a, b; Wimmer et al. 2005). Zn, but not Cd and other transition metals, can facilitate binding of MTF-1 to MRE, and thus activate the MT gene transcription (Bittel et al. 1998). The exact molecular mechanism of action of MTF-1 is poorly understood. Interactions of MTF-1 and other ligands with various REs have been described in more detail in Fig. 2. A general opinion is that Zn binds to an inactive cytoplasmic apo-MTF-1 and activates it by inducing phosphorylation of the protein. The activated complex migrates in the nucleus, where it binds to MRE and stimulates the synthesis of MT mRNA (Smirnova et al. 2000; LaRochelle et al. 2001). An alternative, but

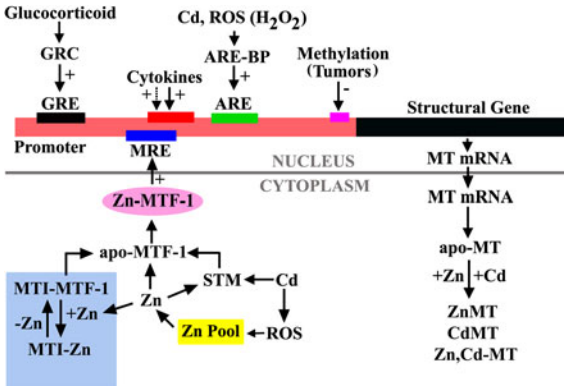

Fig. 2 Schematic presentation of various response elements (RE) in the mammalian (mouse) MT-1 gene promoter and their proposed roles in regulation of gene transcription. Some steps in this scheme (in the *light blue square*) are not clearly supported by experimental findings. *GRE* glucocorticoid RE, *ARE* antioxidant RE, *MRE* metal RE, *MTF-1* metal-regulated transcription factor 1, *MTI* MTF-1 inhibitor, *Zn* zinc, *Cd* cadmium, *ROS* reactive oxygen species (free radicals, such as H_2O_2), *STM* signal transduction molecules, (+) activation, (−) inhibition. GRE can be activated by the glucocorticoid-receptor complex (GRC), whereas cytokines can stimulate their respective RE directly (*arrow*) or via some transduction molecules (*broken arrow*). Apo-MTF-1 is an inactive cytoplasmic protein which, following activation via the Zn-induced phosphorylation, migrates as Zn-MTF-1 into the nucleus to associate with MRE. Cd and ROS can activate MTF-1 indirectly, by mobilizing Zn from the intracellular pool (including from MT itself) and/or can promote activation of MTF-1 via signal transduction molecules (STM). The scheme in *ligh blue square*: according to Palmiter (1994), MTF-1 may exist in inactive form complexed to MTI (MTI-MTF-1); Zn could activate MTF-1 indirectly, by binding to MTI in the complex, and releasing MTI-Zn; the liberated apo-MTF-1 would then be further activated by Zn and bind to MRE. Cd and ROS can activate ARE indirectly, via the ARE-binding proteins (ARE-BP). Methylation in some fast-growing tumor cells, that exhibit a very low content of MT, downregulates the expression of MT-1 gene. Each apo-MT molecule, the final product of the activated MT gene, can bind Zn (ZnMT) or Cd (CdMT) ions, or both (Zn, Cd-MT) (adapted after Davis and Cousins 2000, but including the additional information in the papers by Palmiter 1994; Bittel et al. 1998; Andrews 2000; Smirnova et al. 2000; Vašak and Hasler 2000; Haq et al. 2003)

unconfirmed data by Palmiter (1994) suggested that MTF-1 is kept inactivated in a complex with the Zn-sensitive MTF-1-inhibitor (MTI). Binding of Zn to MTI would release this inhibitor, allowing MTF-1 to be activated by Zn and bind to MRE (Fig. 2, the scheme in the light blue square). Cd and ROS act indirectly, by mobilizing Zn from intracellular stores and/or by activating some other intracellular signaling pathways. None of these ions/molecules are able to activate the MT gene in MTF-1-deficient cells (Heuchel et al. 1994; Dalton et al. 1996c). In a cell free assay, Zhang et al. (2003) demonstrated that apo-MT itself can modulate formation and activity of Zn-MTF-1. This may represent a basic feed-back mechanism that would control the MT production in normal and stressful conditions, but its role in the cells in vivo is not known. Furthermore, the MT gene transcription via ARE can be promoted by various ARE-binding proteins, which can be activated by Cd and ROS. A synergistic activation of the MT gene expression via MRE and ARE may be important in hepatotoxicity and nephrotoxicity induced by Cd and other toxic metals, when the intracellular high concentrations of these metals associate with the increased production of ROS (reviewed in Sabolic 2006).

As opposed to the activating effects of REs on the MT gene expression, DNA methylation, induced by various chemical agents or that occurs in some aggressive tumors, can repress the MT gene transcription by altering chromatin structure, inhibiting binding of activating ligands to their REs, and/or recruiting transcriptional corepressors (reviewed in Haq et al. 2003). Some studies in rats, showing discrepancies in the metal-induced expression of MTs and their mRNAs in liver and kidneys, indicate that the expression of MT can also be regulated at the post-transcriptional level, but this important field has not been well studied (Misra et al. 1997; Klaassen and Lehman-McKeeman 1989; Vasconcetos et al. 2002; Haq et al. 2003).

Localization and roles of metallothionein in intracellular compartments

The presence of MT in all types of mammalian cells suggests that these metalloproteins should play an important role in intracellular functions. However, these roles are not vital for the animal survival and reproduction; neither TG mice with MT-1/MT-2 overexpression nor MT-null mice exhibited major phenotypic or reproductive problems (reviewed in: Palmiter 1998; Vašak and Hasler 2000). The following roles for MT in the mammalian cells have been proposed: (1) homeostasis, storage and transport of essential metals, such as Zn and Cu, (2) Zn and Cu donation to various metalloproteins, transcription

factors, and enzymes; chaperone-like activity for synthesis of various metalloproteins, (3) provision of Zn and Cu during cell differentiation and organ development in prenatal and perinatal periods, (4) protection against metal toxicity by binding toxic metals (scavenging and detoxication) and thus preventing them damaging cellular structures and functions, (5) scavenging free radicals (ROS and RNS) generated in normal metabolism or in oxidative stress induced by various toxic metals and non-metal substances, inflammation or physical injury (anti-inflammatory, anti-oxidant, and anti-apoptotic functions), (6) cell repair and regeneration following partial removal of an organ (hepatectomy) or chemical injury, (7) protection in neurodegenerative and other diseases, and (8) contribution to genotoxicity and carcinogenicity. Various aspects of these proposed functions of MT in the mammalian cells have been extensively discussed in previous expert reviews (Cherian 1994; Moffatt and Denizeau 1997; Palmiter 1998; Suhy et al. 1999; Davis and Cousins 2000; Miles et al. 2000; Nordberg and Nordberg 2000; Vašak and Hasler 2000; Theocharis et al. 2003; Cherian and Kang 2006; Rana 2008; Petering et al. 2009).

Inside the mammalian cells, MT has been detected in specific compartments, e.g., cytoplasm, lysosomes, mitochondria and nuclei (Fig. 3), thus suggesting that it may have some compartment-specific functions in health and disease. A number of studies have indicated a pivotal role of the cytoplasmic MT in homeostasis (trapping, storage, distribution, and release) of Zn and Cu (Tapia et al. 2004; reviewed in: Bremner 1987; Vašak and Hasler 2000; Nordberg and Nordberg 2009; Petering et al. 2009) and in protection from/reduction of oxidative stress induced by toxic metals and related conditions (reviewed in: Andrews 2000; Sabolic 2006; Klassen et al. 2009; Liu et al. 2009; Petering et al. 2009). Having high affinity for Zn and high thermodynamic stability, the cytoplasmic MT represents a potent Zn acceptor and an efficient trap for Zn that enters the cell from the extracellular space via the plasma membrane, from lysosomes following degradation of the Zn-containing molecules, or when liberated from various Zn-containing intracellular structures (Fig. 3A). For this reason, the intracellular concentration of unbound (free) Zn is present in the low picomolar range (Simons 1991). Despite the high stability of

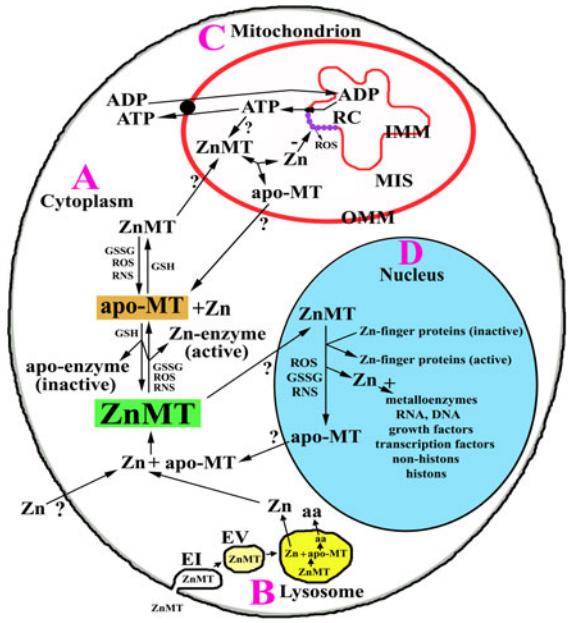

Fig. 3 Roles of MT in Zn homeostasis in various intracellular compartments. MT has been detected in the cell cytoplasm (*A*), lysosomes (*B*), mitochondria (*C*) and nucleus (*D*). In the cytoplasm (*A*), Zn transported from the extracellular space or released from lysosomes following degradation of the endocytosed ZnMT (*B*) binds to apo-MT, forming a pool of ZnMT complex (*green*-labeled). This complex is in equilibrium with metal-free MT (apo-MT; *brown*-labeled) which may represent up to 90% of total MT. The release of Zn from ZnMT or its binding to apo-MT depends on redox reactions, driven by the GSH/GSSG ratio and presence of ROS (see text for details). Liberated Zn binds to and activates (or inhibits) various Zn-dependent enzymes and other metalloproteins. The cytoplasmic ZnMT can enter the mitochondrial intermembrane space (*C*), where liberated Zn inhibits oxidative phosphorylation and limits the production of ROS. The liberation of Zn may be stimulated by ATP binding to ZnMT, but this possibility has not been resolved. In the nucleus (*D*), Zn, liberated from ZnMT, affects the activity of Zn-finger and other metalloproteins important for gene functions. This liberation is stimulated by oxidative conditions, driven by GSSG, ROS and RNS. The question marks denote the poorly known or unknown mechanisms for Zn and ZnMT/apo-MT crossing the plasma, mitochondrial and nuclear membranes, and for ATP and ZnMT interactions. *EI* endocytic invagination, *EV* endocytic vesicle, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *ROS* reactive oxidative species, *OMM* outer mitochondrial membrane, *IMM* inner mitochondrial membrane, *MIS* mitochondrial intermembrane space, *RC* respiratory chain, *RNS* reactive nitrogen species

ZnMT complex, in the cytoplasmic medium Zn can be released and transferred to apo-molecules, and opposite, from the Zn-containing intracellular molecules to apo-MT. This is enabled in redox reactions

that are mediated by reduced (GSH) and oxidized (GSSG) glutathione, and catalyzed by selenium-containing proteins. Thus, GSSG binds to and oxidizes ZnMT, which then enhances release of Zn and its transfer to apo-enzymes, whereas when GSH is prevalent, it binds to and reduces MT, and this condition favors the Zn transfer from enzymes to apo-MT (Jacob et al. 1998; Jiang et al. 1998b; reviewed in: Maret 2003; Kang 2006; Petering et al. 2006). Furthermore, Jiang et al. (1998a) reported that ATP can also bind to MT and decrease its affinity for Zn, thus promoting release of Zn from ZnMT and its transfer to various apo-proteins, but these data were later challenged by Zangger et al. 2000, so that the possible interaction of ATP and MT, which could represent an elegant, energy-related regulator of intracellular MT function, is still an open question. Therefore, the redox status seems to be the major regulator of Zn exchange between MT and other intracellular molecules. The cytoplasmic ZnMT plays a crucial role in fighting oxidative stress, which is characterized by heavily compromised redox status, such as following Cd intoxication in the mammalian liver and kidneys, and in various cultured cells. It has been demonstrated that Cd in these organs/cells: (a) directly inhibits or stimulates the activity of various enzymes (reviewed in Vallee and Ulmer 1972), (b) depletes intracellular anti-oxidants (largely GSH) and free radical scavengers (vitamins E and C), and decreases GSH/GSSG ratio, (c) inhibits the activity of anti-oxidative enzymes (GSH-peroxidase, GSH-reductase, GSH-transferase, catalase and superoxide dismutase), thus shifting the cytoplasmic redox state towards oxidation, characterized by an increased content of ROS and RNS (reviewed in: Kang 2006; Sabolic 2006; Liu et al. 2009; Petering et al. 2009), (d) displaces essential metals bound to MT (Zn, Cu) and various intracellular membranes and metalloproteins (Zn, Cu, Fe); whereas the liberated Cu and Fe, being Fenton metals, promote development of oxidative stress and generation of free radicals in the cells (Casalino et al. 1997; Yiin et al. 1999; reviewed in: Bergendi et al. 1999; Petering et al. 2009), the liberated Zn exhibits potent antioxidative properties by promoting synthesis of new apo-MT via activating MTF-1 and antagonizing Cd binding and reactivity (Andrews 2000; Powel 2000). Besides liberating Zn, the MT molecule itself exhibits redox/antioxidative properties, and: (a) scavenges free radicals and diminishes their oxidizing effects in the cytoplasm, (b) releases bound GSH (and, possibly, ATP), which combats production of free radicals (and, possibly, ATP depletion) in the cytoplasm, and (c) translocates Zn into mitochondria and nucleus (vide infra) (reviewed in: Shaikh et al. 1999; Andrews 2000; Powel 2000; Thevenod 2003; Kang 2006; Petering et al. 2006; Sabolic 2006; Klaassen et al. 2009; Liu et al. 2009; Petering et al. 2009). A finding of significant intracellular concentrations of apo-MT, and different proportions of the metal-saturated and metal-free MT in various cultured cells, tumors, and tissues (Pattanaik et al. 1994; Yang et al. 2001; reviewed in Petering et al. 2006), indicates that the metal-free form of MT may be an important player in various MT activities in the cell health and disease, but these functions have yet to be defined in future studies.

Following its internalization by endocytosis from the extracellular space, MT finishes in lysosomes (Fig. 3B). Inside the acidic compartment, the metal is liberated, the apo-MT is hydrolysed (vide supra), and the final products are then translocated into the cytoplasm by specific transporters. A typical example for this pathway is internalization and processing of CdMT in the renal PT cells (vide infra). We do not know of any specific role for MT inside the lysosomes, although one could imagine that the redox properties of metallo- and apo-protein, before it becomes significantly degraded, could play a role in modulating redox reactions in this highly catabolic medium.

In mitochondria from liver cells (Fig. 3C), MT has been detected in the mitochondrial intermembrane space (Ye et al. 2001). It is assumed that MT in mitochondria arrives from the cytoplasm in form of ZnMT and/or CuMT that cross the outer mitochondrial membrane by unknown mechanism/s. By supplying Zn and Cu, in mitochondria this metalloprotein modulates the activity of Zn- and Cu-dependent metalloenzymes and regulates the rate of respiration. Accordingly, Zn, whose release from ZnMT may be accelerated and enhanced by binding ATP or GTP to the metalloprotein complex (Jiang et al. 1998a; however, see Zangger et al. 2000), inhibits the activity of respiratory enzymes (Jiang et al. 1998b; Ye et al. 2001) and in this way limits respiration and production of ROS. In Cd intoxication, however, mitochondria are direct targets for Cd.

The metal rapidly accumulates in this organelle, possibly via a Ca^{2+} uniporter in the inner mitochondrial membrane (Lee et al. 2005), binds to the electron transfer proteins, and uncouples oxidative phosphorylation, causing hyperproduction of ROS (Tang and Shaikh 2001; Wang et al. 2004a, b). In this toxic condition, mitochondria are not only the major producers of, but are also the major target for ROS. A massive production of ROS compromises metal binding and redox functions of the mitochondrial MT, and impairs the production of ATP. At some point, the overall energy-producing and metabolic breakdown can activate the mitochondrial proteins-mediated intracellular signaling cascade leading cells to apoptosis and/or necrosis (reviewed in: Susin et al. 1998; Kourie 1998; Padanilam 2003; Turrens 2003; Wang et al. 2004a, b; Brookes et al. 2004; Sabolic 2006; Thevenod 2009).

In the nucleus (Fig. 3D), Zn is needed for normal function of histone- and non-histone metalloproteins, nucleolar RNA, metalloenzymes (RNA and DNA polymerases), growth and transcription factors, and particulary for Zn-finger proteins that regulate the activity of various genes; MT represents the major supplier of this essential metal (reviewed in: Wlostowski 1993; Cherian 1994; Petering et al. 2009; Xie and Kang 2009). A recent study by Itoh et al. (2008) revealed that some nuclear transcription factors involved in cell proliferation are also Cu-dependent, but the possible role of MT in transporting Cu into the nucleus is not clear (Ogra and Suzuki 2000; Petering et al. 2009). As found in numerous studies in vivo and in vitro, the livers of adult animals and humans have a very low content of nuclear MT, whereas the fetal and neonatal livers, as well as the differentiating and growing animal and human cells in culture, exhibit a high content of MT in their nuclei (Nishimura et al. 1989a; Cherian and Apostolova 2000; reviewed in Cherian 1994), thus reflecting an increased need for essential metals in rapid cell growth and repair processes. The nuclear MT originates from cytosol, but the exact mechanism of its translocation from the cytosol into the nucleus (and opposite) is not clear. With its molecular mass of $\sim$7 kDa, MT is small enough to pass through nuclear pores, but some studies showed that the cytoplasmic molecule has to be activated by an un-identified cytosolic factor, and that it needs an intact perinuclear cytoskeleton, ATP, a small G protein, and MT mRNA to be transported and retained in the nucleus (Levadoux et al. 1999; Nagano et al. 2000; Takahashi et al. 2005). A trigger for MT translocation may be the general redox state in the cell; it is enhanced in various oxidative stress-related situations with elevated ROS/RNS production, whereas an increased concentration of GSH decreases the nuclear MT. In the nucleus, ROS, RNS, and GSSG enhance liberation of Zn from ZnMT and its transfer to various nuclear proteins that counteract oxidative conditions, and in this way the ZnMT/apo-MT pair also contributes in regulating redox reactions (reviewed in: Cherian 1994; Davis and Cousins 2000). In Cd intoxication, the rate of MT mRNA transcription is strongly enhanced, but delivery of Zn and Cu in a complex with MT is heavily perturbed; the cytosolic MT is preferentially occupied with Cd. In the nucleus, Cd binds to GSH and various Zn-dependent metalloproteins, inhibiting their anti-oxidative and other functions, and causing other toxic events and DNA damage, with apoptosis or cancerogenesis as the possible final effect (vide infra).

Metallothionein in mammalian tissues; age, sex, and strain differences

MT has been detected in various organs in humans and experimental animals, with highest concentrations in liver, kidney, pancreas, and testis (Shimada et al. 1997; Onosaka and Cherian 1981; Danielson et al. 1982a; Palmiter 1987; Onosaka et al. 1988; Nishimura et al. 1989b). The role of MT in handling Cd (and other metals) in liver, kidneys, and in other organs in humans and experimental animals of different age and sex has been poorly investigated. As mentioned previously, very high concentrations of MT, especially in nuclei, were found in liver, kidneys, and other organs during embryonic, fetal and early postnatal periods in both experimental animals and humans, in differentiating and fast growing cells in vitro, and in various tumors (reviewed in Cherian 1994). After birth, during lactation, the levels of MT in major rodent organs were found to be high (Solaiman et al. 2001), and gradually decreased, reaching the adult values after 2–3 weeks (Andrews et al. 1984; Chan and Cherian 1993). In the adult organs, variable amounts of MT were mainly detected in the cell cytoplasm. A high

expression of MT in newborn rats made them tolerant to an acute, hepatotoxic effect of Cd (Goering and Klaassen 1984a, and references there in). However, in the study on human kidneys from 13 weeks gestation through adulthood, MT was detected exclusively in the proximal tubule (PT) cell cytoplasm and nuclei with variable intensity, but no correlation with the stage of development was observed (Mididoddi et al. 1996). Contrary to the relatively well studied expression of MT in the liver and kidneys of prenatal, perinatal, and young adult animals and humans, the expression in aging animals and humans has been poorly investigated. It is assumed that the capacity of renal tissues to produce MT may be decreasing in old age due to less efficient protein synthesis (reviewed in Nordberg 1998). However, in the report by Yoshida et al. (1998), both the liver and kidneys of Japanese people exhibited decreasing levels of MT during infancy and a tendency to increasing tissue content of MT and Cd in the old age. Furthermore, in adult rats and mice the concentration of MT in the liver and kidneys exhibited sex and strain differences. Female organs showed a 2–3-fold higher content of MT than male organs, and this was associated with a significantly greater concentration of Cd in female organs, while very young and very old animals showed no sex differences in MT expression (Blazka et al. 1988; Blazka and Shaikh 1991; Shaikh et al. 1993; Miles et al. 2000). Our own preliminary data indicate that the abundance of MT in the rat kidneys is sex-dependent (males < females) after puberty, and declines in old age (Sabolic et al., unpublished). The origin of these sex differences has not been resolved. Estradiol may stimulate the MT production, but recent findings in isolated rat hepatocytes indicated that progesterone can stimulate Cd influx via activating the verapamil-sensitive Ca^{2+} channels in the hepatocyte membrane; the increased intracellular Cd in female organs may stimulate production of MT, which then can sequester more Cd (Baker et al. 2003). A verapamil-inhibitable uptake of Cd in the human hepatic cell line has already been described earlier, by Souza et al. (1997). However, the presence of verapamil-sensitive L-type voltage operated Ca^{2+} channels in the mammalian liver is still controversial (Khan et al. 1998; Okamoto et al. 2001), so that the mechanism of verapamil action upon Cd and Ca^{2+} transport, and MT expression in hepatocytes, remains to be resolved.

It is interesting that female mice, TG for MT-I, exhibited 4–5-fold higher MT levels in the liver than male mice (Iszard et al. 1995). Although sex differences for MT in the human kidneys have not been documented, women exhibited higher Cd concentrations in blood, kidney cortex and urine, probably due to higher intestinal absorption of Cd, which was inversely related to the body Fe status (Berglund et al. 1994; reviewed in: Järup et al. 1998; Åkesson et al. 2002; Vahter et al. 2007; Nordberg and Nordberg 2009). With its antioxidative activity, a higher MT content in the female kidneys may be related to the well known renoprotective effects of estrogens in experimental models of acute and chronic renal injury in rats and, possibly, in human nephropathies associated with an increased production of ROS (reviewed in: Dubey and Jackson 2001; Kher et al. 2005). Both mice and rats also exhibit strain differences in the liver and kidney expression of MT and sensitivity to Cd toxicity; the strains with more MT in their organs showed less severe $CdCl_2$-induced hepatotoxicity (Shaikh et al. 1993; Mesna et al. 2000; Kuester et al. 2002).

Metallothionein in Cd-induced organ toxicity

A large number of studies have described various aspects of MT in protection from acute and chronic Cd toxicity, and its role in these conditions. Cd is an environmental and occupational hazard that causes damage of the target cells, mainly in liver and kidneys (reviewed in: Hutton 1983; Järup et al. 1998; Järup and Åkesson 2009). However, Cd is largely a man-made occupational and environmental problem, and it is unlikely that the cells had been evolutionary primed to use MT in fighting Cd invasion and toxicity. The role of MT in these actions is not Cd-specific; it is mainly related to the ability of MT: (a) to bind various metal ions, but Cd binds with higher affinity and forms a relatively inert and slow-degrading CdMT complex (vide supra), which is sequestered in the cell cytoplasm and prevents the immediate toxic effects of the cationic (free) Cd, and (b) to scavenge highly reactive ROS/RNS molecules, generated during the Cd-induced oxidative stress, thus diminishing their cytotoxic effects. The ability of a variety of free radical scavengers and antioxidants to prevent, attenuate, or ameliorate the cytotoxic

actions of Cd (and other toxic metals) in various experimental models, indicates that the oxidative stress and the generated ROS/RNS may be the major mediators of cytotoxicity, and that scavenging ROS/RNS and diminishing oxidative stress may be the major role of MT that can be critical for cell survival in these conditions (reviewed in: Andrews 2000; Sabolic 2006; Klaassen et al. 2009). However, the experimental data have indicated that, on one side, MT plays this pivotal role in prevention and protection against Cd-induced toxic effects, but on the other side, it can be a mediator of Cd toxicity.

Role of metallothionein in Cd absorption and toxicity in lungs and intestine

As shown in numerous experimental and epidemiological studies, the main entry pathway for Cd into the mammalian body are lungs and gastrointestinal tract; skin plays a minor role in this respect (reviewed in: Järup et al. 1998; Nordberg 2009; Järup and Akesson 2009). The fate of Cd in the mammalian body following its contact with the lung and intestinal epithelium is schematically shown in Fig. 4.

In the lungs, the source of Cd is usually the inhaled air contaminated with dust, fumes and/or smoke (cigarette smoke!) that contain Cd. In acute intoxication with Cd fumes or dusts with high metal concentrations, where lungs are the primarily affected organs and react with acute irritation, inflammation, edema, and haemorrhage, the basal levels of MT do not play a significant role as a protective or contributing factor. However, if rats were pre-exposed to aerosols with low concentration of Cd, the MT concentration in the lung tissue increased about 50-fold, and enabled good protection from the subsequent cytotoxic doses of Cd (Hart et al. 1989). When lungs are chronically exposed to low metal concentrations, the mechanism of Cd transport into the lung epithelium has not been clearly demonstrated; two transporters, ZIP-8 (Fujishiro et al. 2009) and divalent metal transporter DMT-1 (Ghio et al. 2005) may be involved, but their exact role and localization in the lung cells have not been reported. Within the cells, Cd can bind to the existing MT, and can induce synthesis of new MT. Adult rats exhibit less MT in the lung cells than mice, and this phenomenon may explain a higher resistance of mice to toxic and carcinogenic actions of Cd (Hart et al. 1995; Kenaga et al. 1996; McKenna et al. 1998). MT in the lung cells may play a significant role as a ROS scavenger in protection and survival from oxidative injury by various inducers; MT-null mice exhibited much higher senstivity to oxidative stress (Hart et al. 2001; Takano et al. 2004; Wesselkamper et al. 2006). Possible roles of MT in Cd uptake in, and/or extrusion from the lung cells have not been reported. Modes of release of Cd from the apoptotic and/or oxidative stress-damaged lung cells into the circulation, in the form of cationic (free?) Cd and/or CdMT,

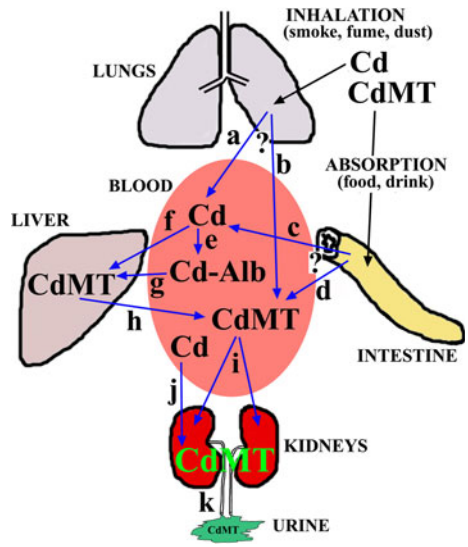

Fig. 4 Pathways of Cd traffic in the mammalian body. Cd can enter the body by inhalation via the lungs or can be absorbed via the intestine. From both organs, it enters the blood by unresolved mechanisms (?), possibly as cationic Cd (*a*, *c*) or bound to MT (*b*, *d*) and/or to some other binding molecules. In the blood, Cd may exist partially as cationic (free) ion, and largely bound to albumin (*e*; Cd-Alb) and other binding proteins (not shown), and partially as CdMT. Both Cd (*f*) and Cd-Alb (*g*) can be internalized by hepatocytes, where Cd-Alb is degraded. In hepatocytes, the liberated Cd stimulates production of apo-MT and binds to it, thus forming new CdMT in the cell cytoplasm. CdMT from the intoxicated liver cells is released into the circulation (*h*), and reaches the renal PT lumen by glomerular filtration, where it is endocytosed and degraded in lysosomes (*i*). Free Cd from the blood can enter the renal tubule cells, possibly by molecular and/or ionic mimicry, e.g., by using specific organic ion and/or metal ion transporters as mediators (*j*). In the renal epithelium, Cd that had been transported and liberated from the degraded CdMT stimulates production of new apo-MT and forms new CdMT, which accumulates in the cell cytoplasm. A portion of accumulated CdMT is continuously released into the urine by unresolved mechanisms, and can be used as a biomarker of Cd nephrotoxicity (*k*)

are also not clear. Furthermore, the cystic fibrosis transmembrane conductance regulator CFTR and the multidrug resistance protein MRP-1, which are present in the lung epithelium (Torky et al. 2005; Regnier et al. 2008), are known to export glutathione (GSH) (reviewed in: Cole and Deeley 2006; Childers et al. 2007), but their possible contribution in Cd-induced toxicity as the exporters of Cd-GSH conjugate into the blood, has not been studied.

In the intestine, Cd arrives via contaminated drink and food. The usual foodstuffs, such as meat (especially the liver and kidney tissues) and plants can contain a variable content of the in vivo accumulated Cd, mainly bound to various reactive molecules, such as glutathione (GSH) and MTs in the animal tissues, and phytochelatins in the plant tissues (reviewed in: Järup et al. 1998; Freisinger 2008; Nordberg 2009; Järup and Akesson 2009). The intestinal absorption of Cd is relatively small, and accounts for 5–8% of the load. Possible mechanisms of Cd transport in the intestinal epithelium have been recently reviewed (Zalups and Ahmad 2003; Bridges and Zalups 2005). Cd seems to be absorbed by enterocytes, mainly in the duodenum and proximal jejunum. At the luminal side, Cd may cross the epithelial cell membrane by ionic mimicry, using several possible transporters for ions, such as the divalent cation transporter DMT-1, Ca^{2+} channels, and by molecular mimicry, in a complex with thiol-containing amino acids and small peptides (cysteine, GSH). However, thus far only DMT-1 has been experimentally proven to mediate active transport of Cd across the apical membrane in duodenal enterocytes (Gunshin et al. 1997). A possible role of MT in Cd absorption at the enterocyte luminal domain is unclear. A study by Moltedo et al. (2000) in cultured intestinal human cell line Caco-2 showed that the cytosolic MT in these cells can be actively secreted; the released MT can bind the ambient Zn (and possibly, Cd), and the metal-MT complex could be re-internalized, possibly by endocytosis, followed by degradation in lysosomes. A contribution of such mechanism in the in vivo intestinal absorption of Zn and Cd, is not known. However, the intestinal enterocytes exhibit a high turnover rate, and MT (integral or truncated molecules) from the damaged and degraded cells may bind Zn and Cd, and be endocytosed by healthy epithelium. In the cells, Cd largely binds to MT (Lehman and Klaassen 1986), whose primary function may be to store and regulate the intracellular Zn concentration (Szczurek et al. 2001, 2009). The mode(s) of Cd transport across the basolateral cell membrane are unclear (reviewed in Bridges and Zalups 2005). Besides temporarily sequestering Cd in the cell cytoplasm, a possible role of MT in Cd transepithelial transport in form of CdMT, via exocytosis/transcytosis, and its delivery to other organs via the circulation has also been considered. Some observations indicated that, following oral Cd administration, Cd absorption in the intestine, and distribution of the ingested Cd into liver and kidneys, was independent on the intestinal MT levels (Liu and Klaassen 1996; Liu et al. 2001a, b). However, a few other studies in mice showed that, following oral ingestion of $CdCl_2$ or CdMT, a variable amount of the CdMT complex can be later found in kidneys (Eisenhans et al. 1997; Cherian et al. 1978; Cherian 1979, 1983; Kimura et al. 1998). This translocated CdMT can originate from transcytosis of the ingested CdMT, from an active extrusion (exocytosis) of CdMT from the intestinal cells at their basolateral side, and/or from release of the complex into the circulation from the cells damaged by ROS following internalization of Cd/CdMT. Recent findings that ionic Cd can disrupt gap junctions between hepatocytes in mice (Jeong et al. 2000), and that it can target E-cadherins and disrupt junctional complexes between epithelial cells in various cell cultures (Prozialeck 2000) and rat PT (Prozialeck et al. 2003), indicate that in the above-mentioned experiments a part of the ingested CdMT may have entered the circulation by crossing the intestinal epithelium paracellularly.

Metallothionein in the blood and its role in Cd traffic

In the blood of humans and experimental animals, most of Zn and Cd is probably bound to serum albumin (the most abundant blood protein), which exhibits low capacity/low affinity binding sites for these metals (Perkins 1961), whereas MT is present only in limited concentrations. The concentration of MT in the blood and in different organs in various species is difficult to compare due to different methods used for determination; a need for optimization and standardization of the relevant method(s) has been announced (reviewed in Nordberg 1998). In

healthy humans, not occupationally exposed to Cd or related toxicants, the blood MT concentration ranged between 0.01 and 1 ng/g serum (Nordberg et al. 1982; reviewed in Nordberg and Nordberg 2009) or 0.51–1.86 ng/ml plasma (Milnerwicz et al. 2009). In healthy humans and intact animals, MT in the blood plasma may represent a high affinity/high capacity transporter of essential metals Zn and Cu among organs, but recent studies have indicated possible functions of the circulating MT in wound healing, inflammation, and immune response (reviewed in Lynes et al. 2006). The sources of MT in blood may be: (a) disintegrated blood and vascular endothelial cells due to senescence, apoptosis and/or necrosis, (b) protein leakage through the cell membrane of cells damaged by oxidative stress and injury, and (c) unconventional loss of the cell content, such as membrane blebbing and formation of exosomes, and (d) regulated secretion of apo-MT/ZnMT, as proposed for cultured human intestinal enterocytes (Moltedo et al. 2000) and mouse adipocytes (Trayhurn et al. 2000).

Following absorption in the lungs and/or intestine, it is assumed that Cd in the blood primarily binds to albumin and other thiol-containing reactive biomolecules in the plasma, whereas CdMT may be transported unchanged. In occupationally exposed male workers to Cd, the plasma MT was ~10-fold higher (2–11 ng/g plasma) than in unexposed workers (0.01–1 ng/g serum) (Nordberg et al. 1982; reviewed in Nordberg and Nordberg 2009), whereas in rats and rabbits chronically treated with $CdCl_2$, the plasma CdMT increased progressively with the duration of treatment and in parallel with the plasma Cd concentration, indicating that blood is the medium that delivers CdMT from the affected organs (mainly liver) to the kidneys (Shaik and Hirayama 1979; Tohyama and Shaikh 1981; Nomiyama et al. 1998; Dudley et al. 1985). However, red blood cells (Rahman et al. 2000), lymphocytes (Lu et al. 2005), and platelets (Sagiura and Nakamura 1994) also contain MT, which is inducible by Cd, and may also contribute to the distribution of CdMT via the systemic circulation. The expression of MT-I/II mRNA in blood and peripheral lymphocytes has recently been used as a sensitive biomarker of environmental and occupational exposure to Cd in human population (Lu et al. 2005, 2007; Chang et al. 2009).

Role of metallothionein in acute and chronic Cd-induced hepatotoxicity

Cd circulating in blood as a cationic form (free ion? probably at a very low concentrations) and/or largely bound to albumin, microglobulins, α-2-macroglobulin, and other SH-containing biomolecules, can enter hepatocytes by poorly defined mechanisms (reviewed in: Zalups and Ahmad 2003; Bridges and Zalups 2005; Watkins et al. 1977). Free Cd may cross the sinusoidal membrane by ionic mimicry via DMT-1, ZIP-8, and/or verapamil-sensitive Ca^{2+} channels, whereas Cd-albumin and similar complexes may be internalized by endocytosis and degraded in lysosomes (Baker et al. 2003; Fujishiro et al. 2009; Souza et al. 1997), but the relative contribution of these pathways is controversial (Pham et al. 2004). Whether CdMT can enter hepatocytes is not clear. When male rats were injected i.v. with the radiolabeled $CdCl_2$, most of the Cd (~65% of the dose) ended in the liver, and only a small percentage was found in the kidneys (~2% of the dose) and other organs. In rats injected with radiolabeled CdMT, the largest portion of the dose was measured in the kidneys (~40%), whereas the liver contained much less (~11%) of the dose (Cherian et al. 1976). In an in vitro study in human hepatocellular carcinoma cells, CdMT was internalized similarly to albumin, via lipid raft (caveolae)-dependent endocytosis (Hao et al. 2007). However, it has never been shown whether hepatocytes can indeed internalize CdMT in vivo. To address this problem, we have injected rats i.v. with CdMT, fixed their organs with 4% *p*-formaldehyde by perfusion in vivo at different time points following injection, and immunostained tissue cryosections for MT (experimental details described in Sabolic et al. (2002)). As shown in Fig. 5, 15 min following CdMT injection, some hepatocytes exhibited a variable amount of endogenous MT, homogeneously distributed in their cytoplasm and nuclei (this pattern was also observed in tissue cryosections from control, saline-injected animals; c.f. Fig. 6D), but only the Kupffer cells strongly accumulated the injected CdMT in intracellular organelles (probably endosomes). None of the hepatocytes exhibited any visible endosome-like staining even 2 h following the injection of CdMT (data not shown), while in the renal PT cells of the same animals, a time dependent endocytosis of CdMT was dramatic (c.f., Fig. 8).

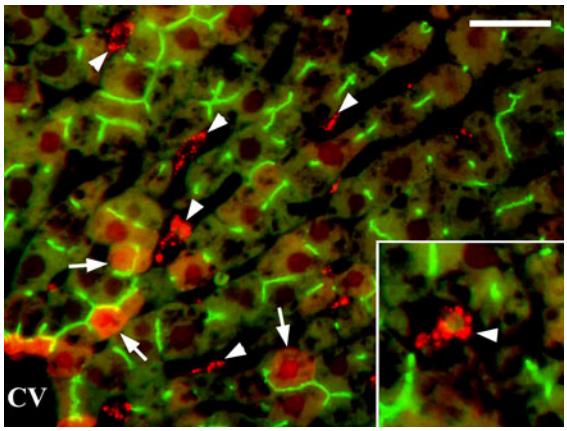

Fig. 5 Immunolocalization of MT in the rat liver 15 min following i.v. injection of CdMT (a single dose of 0.4 mg Cd/kg b. m.). In hepatocytes, MT (*red fluorescence*) was diffusely but heterogeneously stained in the cytoplasm and nuclei of some cells (*arrows*), but most hepatocytes exhibited no visible staining at all. This staining pattern was identical to that in the liver of control rats, which had not been injected with CdMT (c.f., Fig. 6D). However, the Kupffer cells, which were not stained at all in the control liver (c.f., Fig. 6D), strongly accumulated the injected CdMT complex in intracellular organelles, most probably endosomes (*arrowheads*, and *inset*). None of the hepatocytes exhibited any endosome-like staining, indicating an absence of significant endocytosis of CdMT. The green fluorescence denotes bile canaliculi, stained with an antibody against cell adhesion molecule CAM-105. CV, central vein; *bar* 20 μm

Therefore, unlike the situation in human hepatocellular carcinoma cells in vitro, our experiment indicates that CdMT is an unlikely substrate for rat hepatocytes in vivo. Since previous studies have shown that endocytosis of CdMT in renal PT cells was mediated by the scavenger receptor megalin (Klassen et al. 2004; Wolff et al. 2006), we have applied different unmasking techniques to detect megalin in tissue cryosections of the rat liver, but without any success (Sabolic et al., unpublished). Therefore, any possible uptake of CdMT in rat hepatocytes in vivo could be very small and megalin-independent. In the Kupffer cells, the endocytosed complex may be degraded and Cd can be subsequently released and then taken up by hepatocytes via ionic transport. Alternatively, Cd may activate the Kupffer cells to release various inflammatory interleukins, which can stimulate production of ROS and MT in hepatocytes (reviewed in Liu et al. 2009).

In rodents, Cd is a potent hepatotoxin after acute or chronic exposure. Inside the hepatocytes, Cd principally binds to cytoplasmic proteins, largely MT, and thus becomes inactivated and trapped inside the cytoplasm. The experiments in primary cultured rat hepatocytes have shown that about 50% of the cellular Cd is loosely bound whereas the other 50% is firmly bound to intracellular components (Pham et al. 2004). Due to high affinity of MT for Cd, and due to high stability (slow degradation) of the complex at the cytoplasmic pH, as well as due to Cd-stimulated synthesis of new apo-MT, the cytoplasmic CdMT accumulates with time and can reach high concentrations. During a short-term poisoning with high Cd doses in experimental animals, it is assumed that the burden with (cationic/free?) Cd eventually becomes higher than the binding capacity of the existing MT in hepatocytes, causing an acute and lethal liver injury due to an extensive oxidative stress and ROS/RNS-mediated damage of the cell structure and function, manifested by inflammation, apoptosis and/or necrosis of hepatocytes. The lethality of hepatotoxic doses of Cd was: (a) absent in immature rats, which in the liver exhibited 10–20 times higher concentrations of MT than the adult animals (Goering and Klaassen 1984a, b) prevented by pretreating animals with small doses of Cd that induce synthesis of MT in hepatocytes (Goering and Klaassen 1984b, c) absent in mice TG for MT-1, which exhibit 10–20-fold higher levels of this protein in the liver (Liu et al. 1995), (d) increased in MT-null mice (Masters et al. 1994), and (e) diminished in rats pretreated with Zn, an approach known to upregulate MT concentration in the liver and other organs (Goering and Klaassen 2004c). These data, therefore, indicate strong protective and anti-oxidative functions of MT in acute Cd-induced hepatotoxicity.

In chronic Cd-hepatotoxicity, which in mice, rats, and rabbits can be induced with repeated small doses of $CdCl_2$ for a few weeks to few months, MT and several antioxidants were also found to protect from the injury induced by oxidative stress and ROS (Nomiyama et al. 1998; Shaikh et al. 1999; Habeebu et al. 2000a). However, in various experimental models, a clear-cut generation of oxidative stress and ROS following long term treatment with small Cd doses was not always observed, possibly due to slow development of the processes and activation of various compensatory defense antioxidative mechanisms in the cells, such as a rise in MT and GSH levels (reviewed in Liu et al. 2009). The long-term

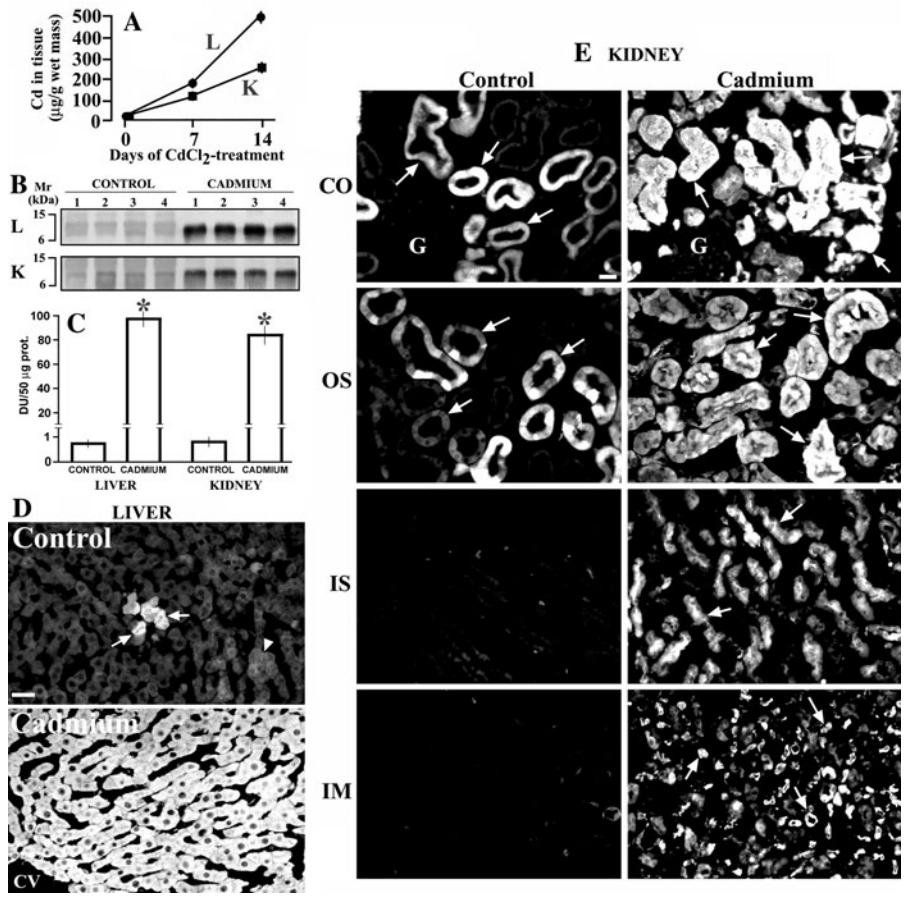

Fig. 6 Expression of MT in the liver and kidneys following subchronic treatment of rats with $CdCl_2$ for two weeks. **A** Accumulation of Cd in the liver (*L*) and kidney (*K*) tissue following daily treatment of rats with $CdCl_2$ (2 mg Cd/kg body mass, s.c.) for up to 14 days. After 2 weeks of treatment, the concentration of Cd in the liver wet tissue (~500 μg/g) was about twice as high as in the kidneys (~260 μg/g). **B** Western blots of MT in the tissue extract (post-19,000×*g* supernatant from the tissue homogenate) from the liver (*L*) and kidneys (*K*) of vehicle-treated (*control*) and $CdCl_2$-treated (Cadmium) rats. The MT-related 7–8 kDa protein band was strongly enhanced in rats treated with $CdCl_2$ for 2 weeks. **C** Densitometric evaluation of the bands shown in (**B**); in $CdCl_2$-treated rats, the band density in the liver and kidney tissues increased ~135-fold and ~95-fold, respectively (* vs. control, $P < 0.001$). **D** Immunolocalization of MT in the liver from vehicle (control)- and $CdCl_2$-treated (Cadmium) rats. In control rats, the individual or grouped cells were weakly (*arrowhead*) or strongly (*arrows*) stained, while most of other cells remained virtually unstained. In Cd-treated animals, all the cells were strongly stained. CV, central vein; *bar* = 20 μm. **E** Immunolocalization of MT in the kidneys of vehicle (control)- and $CdCl_2$-treated (Cadmium) rats. In control rats, MT was heterogeneously stained in the PT segments in the cortex (CO) and outer stripe (OS) (*arrows*), whereas the nephron segment in the inner stripe (IS) and inner medulla (IM) were not significantly stained. In Cd-treated rats, the nephron segments in all tissue zones were strongly stained (*arrows*). In the CO and OS, many tubules were heavily damaged, exhibiting irregular morphology and the presence of cell debris in their lumen. *G* glomerulus; *bar* 20 μm

Cd treatment in experimental animals results in a time- and dose-dependent accumulation of Cd and increase in MT content in the liver. Thus, while the liver MT concentration in control rats was 18–30 μg/g tissue (Onosaka and Cherian 1981; Chan et al. 1993; reviewed in: Webb 1986; Klaassen et al. 1999; Nordberg 1998; Nordberg 2009), following 16-days treatment with different doses of $CdCl_2$, the MT increase was Cd dose-dependent and rose up to 240-fold (Webb 1986). A dramatic effect was also observed in the liver of WT mice acutely treated with $CdCl_2$, when MT from control values (~4 μg/g tissue) increased ~60-fold after 24 h, whereas in MT-null mice, the liver MT was very low and not

upregulated by Cd treatment (Liu et al. 1996a). Our comparable findings by Western blotting in tissue extract, and by immunocytochemistry in tissue cryosections, in rats treated subchronically with $CdCl_2$ (Sabolic et al., unpublished data), are summarized in Fig. 6. This, in vivo model of Cd-toxicity was described by us in details previously (Sabolic et al. 2001, 2006). Accordingly, male rats were treated with either saline (controls) or $CdCl_2$ (2 mg Cd/kg body mass/day) for 2 weeks, and Cd accumulation and expression of MT in the liver tissue were studied (Fig. 6A–D). We measured a time-dependent accumulation of Cd in the tissue (Fig. 6A), and after 14-day-treatment, Western blotting of proteins in the tissue extract revealed a weak protein band of MT in control rats, and ~135-fold stronger band in Cd-treated rats (Fig. 6B, C). By immunocytochemistry (Fig. 6D), many cells in tissue cryosections from control livers were weakly positive for MT, while some individual cells or aggregates of a few cells, exhibited stronger MT expression in both cytoplasm and nuclei. After 14-day-treatment with $CdCl_2$, all hepatocytes were heavily positive for MT.

An important pathophysiological result of either acute or chronic Cd-induced hepatotoxicity is a release of CdMT from the damaged hepatocytes into the blood (Dudley et al. 1985; Nomiyama et al. 1998) and its arrival in the kidneys, where it is filtered and further processed by the PT epithelium.

Role of metallothionein in Cd-induced nephrotoxicity

The long term environmental and/or occupational exposure to small Cd concentrations in humans and animals targets the kidney structure and function, causing defects in reabsorptive and secretory functions with urinary symptoms resembling the acquired Fanconi syndrome. These symptoms indicate that the PT is the major target of Cd. Indeed, in various animal models of chronic Cd-induced nephrotoxicity, either parenteral or oral treatment with various doses of $CdCl_2$ for a few days to few years resulted in a variety of functional defects and structural damage to the brush-border (BBM) and basolateral (BLM) membrane, cytoskeleton, and intracellular organelles of the PT epithelium (reviewed in: Nomiyama 1980; Järup et al. 1998; Sabolic et al. 2006; Järup and Akesson 2009; Nordberg 2009). Such a treatment has been associated with the upregulation of MT content in the kidney tissue; whereas in intact rats the measured tissue content of MT was 30–60 μg/g wet tissue, in rats treated with different $CdCl_2$ doses for up to 16 days the kidney MT concentration increased dose-dependently, up to 23-fold (Onosaka and Cherian 1981; Chan et al. 1993; reviewed in: Webb 1986). Furthermore, an acute $CdCl_2$-treatment in WT mice resulted in ~12-fold increase in renal MT, from 2.4 μg/g in controls to ~28 μg/g 24 h following treatment, whereas in MT-null mice, the tissue MT was negligible and Cd treatment-insensitive (Liu et al. 1996). To support these observations, we recently used the rat model of subchronic Cd-nephrotoxicity, induced with daily injections of $CdCl_2$ s.c. for 2 weeks (methods described in Sabolic et al. 2001, 2006), and correlated the accumulation of Cd with the expression of MT by Western blotting and immunocytochemistry of the kidney tissue (Fig. 6; our unpublished data). As shown in Fig. 6A, the kidneys exhibited a time-dependent accumulation of Cd in their cortex; after 2 weeks, the concentration became higher than the previously defined "critical" concentration for the visible onset of nephrotoxic symptoms in rats (150–250 μg Cd/g wet mass; reviewed in Nomiyama 1980). Furthermore, this treatment strongly upregulated the MT concentration in the renal cortex; after 14-day-treatment, the MT protein band in the tissue extract was weak in saline-treated rats, and increased ~95-fold in density in $CdCl_2$-treated rats (Fig. 6B, C). By immunocytochemistry in tissue cryosections (Fig. 6E), MT in the control kidneys was stained with variable intensity nearly exclusively in the PT segments of the cortex and outer stripe, whereas in the $CdCl_2$-treated rats (Cadmium), the nephron segments in all tissue zones were strongly stained. In the cortex and outer stripe, the lumen of many PT was distended and filled with cell debris, largely due to desquamation of BBM from heavily injured epithelial cells.

Although during Cd intoxication a variety of cells in the body can produce and release CdMT, there is a general view that Cd-nephrotoxicity is primarily induced by the CdMT complex, which is formed in, and released from the Cd-injured liver, and which then reaches the kidney via the circulation (Dudley et al. 1985; Nomiyama et al. 1998; Klaassen et al. 2009; Nordberg 2009). The mechanism of CdMT release from the intoxicated hepatocytes has not been

resolved; the complex may originate from necrotic and/or apoptotic cells, but some kind of regulated secretion, similar to that proposed for apo-MT/ZnMT in cultured human intestinal enterocytes (Moltedo et al. 2000) and mouse adipocytes (Trayhurn et al. 2000), may also be involved. A direct connection of the liver CdMT and kidney injury was observed following transplantation of the Cd-intoxicated rat liver into an healthy rat; CdMT, derived from the donor liver, was released into the circulation and transferred to the acceptor's kidneys, where it induced damage to the previously healthy PT (Chan et al. 1993). However, recent findings indicate that some heavy metals, such as Hg, may enter PT cells by mechanisms that include transports via ionic and/or molecular mimicry across both BBM and BLM, as recently reviewed by Zalups and Ahmad (2003) and Bridges and Zalups (2005), but these mechanisms have been poorly studied, and for Cd are rather hypothetical. The scheme in Fig. 7 shows that in rats the Cd transport via ionic mimicry may include DMT-1 and Ca^{2+} channels in the BBM, whereas in the BLM, Cd bound to *N*-acetylcysteine and/or GSH may cross the membrane by molecular mimicry via organic anion transporters, such as Oat-1 and/or Oat-3. However, localization of DMT-1 in the mammalian proximal tubule BBM is still controversial (reviewed in: Smith and Thevenod 2009), whereas the basolateral transport of Cd via Oats is only an assumption, based on the observation that in the rat renal BLM organic Hg (methyl-Hg) can be transported in a complex with *N*-acetylcysteine by Oat-1 (Koh et al. 2002), and that GSH is a substrate for Oat-3 (Lash et al. 2007). Further studies are needed to resolve these problems. Moreover, in chronic exposure to Cd salts in humans, or in $CdCl_2$-treated experimental animals, a relative contribution to nephrotoxicity of Cd that enters the renal cells as a free cation, as Cd complexed with non-MT molecules, and as the CdMT complex can not be estimated with certainty. This contribution may depend on the experimental model; in intact animals, the parenterally applied CdMT was more nephrotoxic than $CdCl_2$ in spite of higher tissue accumulation of Cd following injection of $CdCl_2$ (Dorian et al. 1995), while the cultured rat PT cells and LLC-PK1 cells exhibited more rapid and much higher uptake and cytotoxicity of Cd from the medium containing radiolabeled $CdCl_2$ than CdMT (Prozialeck et al. 1993; Liu et al. 1994). On the other side, in MT-null mice, an s.c. treatment with $CdCl_2$ for 10 weeks resulted in much worse nephrotoxicity than in similarly treated WT mice (Liu et al. 1998). These data thus indicate that in vivo, circulating CdMT may be a major mediator of

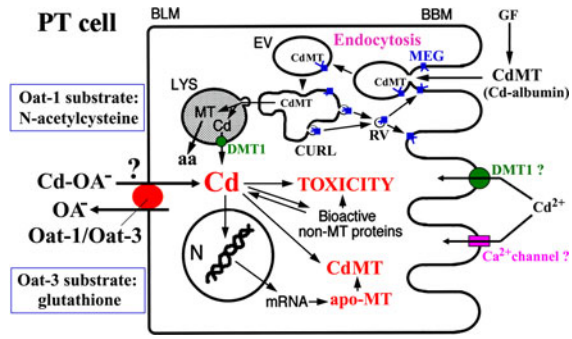

Fig. 7 Fate of CdMT and Cd in the renal PT cell. CdMT reaches the tubular fluid by glomerular filtration (GF), binds to megalin (MEG) at the brush-border bottom, where it is internalized in endocytic vesicles (EV). The endocytic process depends on the vacuolar H^+-ATPase-mediated intravesicular acidification and intact cytoskeleton, and is regulated by small G-proteins (Wolff et al. 2008; reviewed in: Marshansky et al. 1997; Verroust and Kozyraki 2001). The MEG-bound CdMT in EV is delivered to the recycling compartment (compartment for uncoupling receptor and ligand; CURL), where CdMT is released from its receptor. MEG recycles back to the brush-border membrane (BBM) by recycling vesicles (RV), whereas CdMT is delivered to lysosomes (LYS) by an another type of vesicles (not shown). In the lysosomal acidic medium, Cd is liberated, and apo-MT is degraded to amino acids (aa), whereas liberated Cd is transported by the divalent metal transporter DMT-1 into the cell cytoplasm. Since megalin is also a receptor for albumin (reviewed in Christensen et al. 2009), the filtered Cd-albumin may follow the same pattern. Free Cd may cross both cell membrane domains via ionic/molecular mimicry, by exploiting the existing transporting mechanisms for other inorganic ions, such as for Fe^{2+} and Ca^{2+}, or organic anions, such as for *N*-acetylcysteine or glutathione. At the apical/BBM side, the ways of transport of the filtered Cd may be via DMT-1 and/or Ca^{2+} channels, whereas at the basolateral side (BLM), Cd may bind to organic anions (OA^-) *N*-acetylcysteine and/or glutathione, and these complexes ($Cd\text{-}OA^-$) may be translocated into the cell by organic anion exchangers Oat-1 and Oat-3, respectively. The internalized and liberated Cd indirectly stimulates production of MT mRNA and apo-MT, which in the cytoplasm binds Cd (CdMT) and neutralizes its toxic effects. If the Cd load in the cytoplasm is too fast and/or too high, the unbound Cd binds to various bioactive proteins, and causes oxidative stress and other toxic effects. It should be noted, however, that whereas the CdMT-related (endocytic) pathway has been well documented, the other pathways of Cd transport have not been experimentally confirmed. Various modes of Cd transport across the cell membrane have been reviewed in more detail by Thevenod (2010). *N* nucleus

nephrotoxicity in humans and animals chronically intoxicated with Cd salts, but cytosolic Cd liberated from CdMT may be the active component in causing cytotoxicity, while newly synthesized MT can protect from the toxic actions of liberated Cd.

According to the present model for chronic Cd-induced neophrotoxicity (Fig. 7), the circulating CdMT reaches the PT lumen by ultrafiltration in glomeruli. The complex is then internalized by megalin-dependent endocytosis at the bottom of BBM, and delivered to the endo/lysosomal compartment for degradation, whereas megalin recycles back to the BBM (Klassen et al. 2004; Wolff et al. 2006, 2008; reviewed in Thevenod 2010). A relatively fast (in matter of hours) degradation of the i.v. injected CdMT was reported in the mouse kidney in vivo, but the liberated Cd stayed in the cells (Dorian et al. 1992, and references there in). Following degradation in lysosomes, the liberated Cd is transported into the cell cytoplasm, probably via DMT-1 localized in the limiting lysosomal membrane (Abouhamed et al. 2006), where it primarily binds to endogenous cytoplasmic MT and indirectly generates conditions for activation of MTF-1, which then stimulates de novo production of apo-MT (vide supra). It is assumed that in overloading conditions, Cd can bind to other intracellular molecules, induce a significant oxidative stress, with further development of toxic conditions that can end in apoptosis or necrosis (Erfurt et al. 2003; reviewed in: Thevenod 2003; Sabolic 2006). In the cells treated with CdMT, in which the expression of DMT-1 was attenuated by gene silencing, the exit of Cd out of lysosomes was diminished, and the cytotoxicity and apoptosis were decreased, thus supporting the view that the liberated Cd, not CdMT, is the active toxic agent in the PT cells (Abouhamed et al. 2007). In this game, MT protein itself is not nephrotoxic; ZnMT did not produce kidney damage, and was rather protective in nephrotoxicity induced with CdMT (Dorian and Klaassen 1995).

To illustrate the fate of CdMT following its filtration in the kidneys, in rats injected i.v. with this complex we followed the initial, time-related events in endocytosis of CdMT in the PT in vivo, and their relation with megalin. Using specific antibodies (Sabolic et al. 2002, 2006), colocalization of MT and megalin in PT was studied by immunocytochemistry in tissue cryosections (previously unpublished data). As shown in Fig. 8, in the cortical PT, already 5 min following injection, MT (CdMT, red staining) was localized in numerous endosomes beneath the BBM, whereas at later time points, it was found in larger intracellular organelles (lysosomes) deeper in the cell cytoplasm. At 5 and 15 min, the yellow-staining indicated colocalization of both MT (red) and megalin (green) in the same organelles (endosomes), whereas at later time points (at 30 and 60 min), the brightly red-stained large organelles (lysosomes) were full of MT and devoid of megalin. However, at 120 min, in many cortical tubules the clear distinction between intracellular organelles and BBM was lost due to appearance of necrosis in the cells. In the outer stripe, the PT straight segments exhibited overall weaker endocytosis, with the pattern similar to that in the cortical tubules, but without visible cell necrosis at 120 min. Similar pattern of events we also observed in rats treated with CdMT s.c., except that the whole process of endocytosis, accumulation of MT in lysosomes, and related injury in the cortical PT developed later, in the range of hours (Sabolic et al. 2002, 2006). These data led us to assume that the acute and massive accumulation of CdMT in lysosomes following i.v. injection of CdMT has not only one (Cd-induced generation of oxidative stress in the cell cytoplasm), but several immediate deleterious effects on the cell structure and function, which can result in the cell injury and necrosis in a short time, even before generating oxidative stress. These effects are: (a) overloading of the endo/lysosomal system in the PT with the endocytosed proteins, which is known for itself to be inhibitory/toxic to the intracellular vesicle recycling and cell function (reviewed in D'Amico and Bazzi 2003), (b) already small concentrations of liberated Cd can inhibit vacuolar H^+-ATPase (which is important also for acidification and function of lysosomes) and depolymerize microtubules; these effects can diminish the lysosomal function and block the intracellular vesicle recycling and other, acidification-dependent cellular functions (Herak-Kramberger et al. 1998; Sabolic et al. 2002, 2006), and (c) liberated Cd can directly affect and damage the integrity of cell membranes and intracellular organelles (Herak-Kramberger et al. 1998, 2001); the damaged lysosomal membrane can result in leakage of proteolytic enzymes into the cytoplasm and the enzymes can immediately start digesting the cell

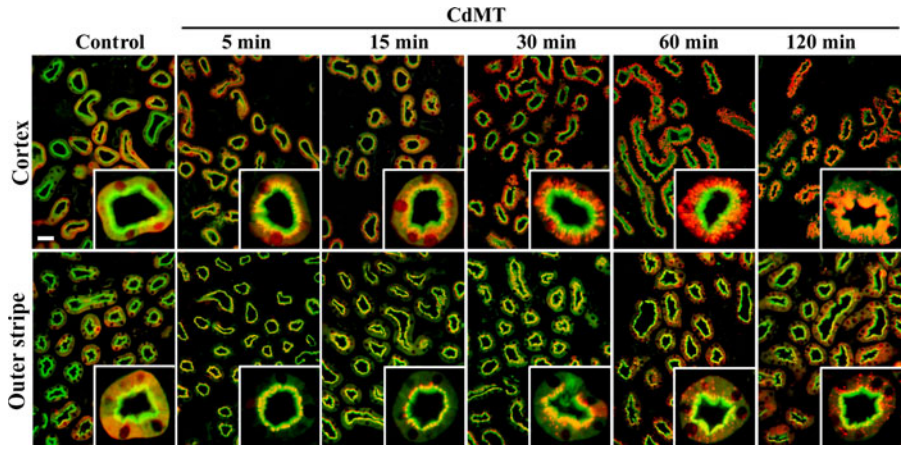

Fig. 8 Time course of endocytosis of CdMT, injected i.v. into rats in vivo (a single dose of 0.4 mg Cd in form of CdMT/kg body mass), in the PT segments of the cortex (proximal convoluted (S1/S2) segments) and outer stripe (S3 segments); double staining of MT (*red*) and megalin (*green*). Control animals were injected with vehicle (saline), and were sacrificed after 120 min. In the tubules of control rats, MT was largely detected as a smooth cytoplasmic staining of heterogeneous intensity in individual cells of both tissue zones, whereas megalin was exclusively localized to the brush-border membrane (BBM). As shown for cortical tubules (Cortex, *larger pictures* and *insets*), already 5 min after i.v. injection of CdMT, the yellow spikes at the bottom of BBM indicated formation of endocytic invaginations containing both red CdMT and green megalin. With time (at 15, 30, and 60 min following injection), the increasingly more *red*-stained organelles with CdMT, but with less green-stained megalin, accumulated in the cell interior. After 60 min, the cells were heavily filled with organelles loaded with CdMT (probably lysosomes), whereas megalin was strictly apical. At 120 min, many tubule profiles showed disappearance of granular and appearance of diffuse intracellular staining, and various kinds of necrotic damage, such as the loss of BBM and tubular structure, which were similar to those observed 12 h after injecting the same dose of CdMT s.c. (Sabolic et al. 2002). In the outer stripe tubules, the pattern of events was similar, except that the abundance of CdMT-positive organelles was at any time smaller, and the tubule damage at 120 min was not observed. *Bar* 25 μm

content. These immediate effects of Cd, liberated following degradation of CdMT in lysosomes, could readily interrupt reabsorption of the filtered proteins (manifested by tubular-type proteinuria), and could lead to the fast-developing functional and metabolic breakdown, and cell death. These immediate phenomena have not been sufficiently considered in previous studies of the acute, CdMT-induced nephrotoxicity.

Contrary to its role in the endocytosed CdMT complex as a carrier for toxic Cd (MT as a mediator of Cd-nephrotoxicity), MT present in the cell cytoplasm has beneficial effects on Cd-induced nephrotoxicity. MT in the PT cell cytoplasm seems to be important for sequestration and accumulation of the liberated Cd, and in this way it protects from, and ameliorates the toxic Cd actions (ROS/RNS scavenger). This is indicated by the following findings: (a) in MT-null mice, the absence of MT did not affect the initial accumulation of Cd following $CdCl_2$ treatment, but the rate of Cd elimination was faster. This means that the cytosolic MT is necessary to bind and sequester/buffer Cd (Liu et al. 1996a). (b). Pretreatment of rats and mice with small doses of $ZnCl_2$ and $CdCl_2$ in order to upregulate MT, protected from the acute nephrotoxicity induced by CdMT (Jin et al. 1987; Min et al. 1987). The Zn ion, however, also exhibited some other, MT-independent protective actions, possibly by stabilizing cellular (and lysosomal?) membranes and tight junctional complexes, by increasing the level of heat shock proteins, and by supplying Zn-finger proteins, including MTF-1, with Zn (Liu et al. 1996b; Jacquilet et al. 2006). (c) MT-null mice were more sensitive to chronic $CdCl_2$-induced nephrotoxicity than the corresponding WT mice (Liu et al. 1998, 1999a, b). However, MT-I TG mice, which have 10-fold more renal concentration of MT-1 than the WT animals, were not protected from the nephrotoxic effects of acute, i.v. injection of CdMT (Liu et al. 1996b). These controversial observations have not been resolved.

Previous studies in humans and experimental animals showed that the initial symptoms of nephrotoxicity following chronic Cd exposure, with

dysfunction of PT, such as tubular (low MW) proteinuria, aminoaciduria, and glucosuria, occur when the level of accumulated Cd in the renal tissue reaches a "critical" concentration. This concentration seems to be species-dependent; for humans, it is estimated at ~200 μg Cd/g tissue, and for rats and mice at 150–250 μg/g tissue (reviewed in Nomiyama 1980). However, a comparison between the three different models of Cd-induced nephrotoxicity in rats, e.g. a chronic treatment with $CdCl_2$, a chronic treatment with small doses of CdMT, and an acute treatment with a high dose of CdMT, revealed that the symptoms of renal injury in chronic models appeared at high concentration of both Cd (85–110 μg/g) and MT (400–600 μg/g) in the renal tissue, whereas in the acute model, it appeared at very low Cd (5–7 μg/g) and MT (40 μg/g) (Liu et al. 1998). Using these data, one can easily calculate that in the chronic model, the concentration of free Cd equivalents exceeds the concentration of Cd bound to MT molecules by ~80%, whereas in the acute model, the concentration of free Cd equivalents exceeds the concentration of Cd bound to MT by only ~35%. These data clearly support the sequestering and protective role of the newly synthesized MT during chronic Cd intoxication, and that Cd may become toxic in overloading conditions only, e.g., after a certain amount of free Cd overcomes the buffering capacity of cytoplasmic MT. Further Cd-related developments of oxidative stress and intracellular events in the mammalian kidneys have been reviewed in more detail previously (Shaikh et al. 1999; Thevenod 2003; Sabolic 2006; Klaassen et al. 2009; Liu et al. 2009; Thevenod 2009).

Metallothionein in the urine

MT in the urine is closely linked to urine Cd which exists mainly as CdMT. Again, different methods of determination and quantification in different studies gave very different levels of urinary MT. When measured by the same laboratory with a RIA assay, in non-smoking humans, Cd in the urine seems to be related to Cd intake, age and sex (Tohyama et al. 1981a; Olsson et al. 2002; Omarova and Phillips 2007). Thus, the control middle-aged human males had urine MT of ~150 μg/g creatinine; this parameter was ~1.2-fold higher in the long-term smelter workers (Tohyama et al. 1981a), and ~2.6-fold higher in an older female population living in a non-polluted area (Tohyama et al. 1981b). However, much higher values of urine MT were found in a population living in a Cd-polluted area and in Cd-intoxicated patients with "itai-itai" disease (Tohyama et al. 1981b; reviewed in Nordberg and Nordberg 2009).

Some studies in experimental animals and humans have suggested that urine Cd might represent a marker for total Cd body burden. However, recent studies in mice chronically treated with low, environmentally relevant concentrations of Cd in drinking water, showed that the urinary Cd levels can not be correlated with either blood, liver or kidney Cd content; rather, the kidney Cd content may be a reliable indicator of the total body burden in chronic Cd exposure (Thijssen et al. 2007). On the other side, recent studies in humans have indicated urine MT as a more perspective indicator/biomarker for monitoring environmental and/or occupational Cd-exposure and associated kidney damage. In some studies, urine MT correlated well with the Cd concentration in the kidney tissue but not with that in the blood (Tohyama et al. 1981b; Nordberg et al. 1982; Roels et al. 1983). Moreover, urine MT in humans exhibited sex differences (females > males), which also correlated with the urine concentrations of Cd and Zn (Folch et al. 1998). However, a more recent study in a Chinese population by Chen et al. (2006a, b, c) showed an excellent correlation of urine MT with blood and urine Cd in non-smokers and cigarette-smokers in occupationally non-exposed and exposed groups, which suggests that urinary MT could reflect the increase of Cd body burden, and that MT can be a better indicator/biomarker of renal dysfunction than commonly used urinary beta-2-microglobulin (Chen et al. 2006b).

The experiments in Cd-treated rats showed that the urine MT gradually increased with the duration of treatment (Shaikh and Hirayama 1979; Tohyama and Shaikh 1981; Tohyama et al. 1981a; Sugihira et al. 1986). However, while the urine CdMT in Cd-treated animals and environmentally or occupationally Cd-exposed humans may originate from the damaged tubule epithelium (that underwent apoptosis and/or necrosis), the source of MT in non-exposed animals and people is less clear. This source may be MT complexes with Zn, Cu, or Cd that are: (a) filtered

from the blood, but escaped endocytosis along the nephron, (b) released from tubule cells due to higher membrane permeability caused by ROS generated during normal metabolism, (c) released from tubule cells that underwent apoptosis driven by the normal cell cycle (senescent cells?), (d) released during shedding of the cell membrane as a result of a continuous membrane remodeling, and (e) released by one of the nonclassical modes of protein secretion, such as membrane blebbing and formation of exosomes (reviewed in: Nickel 2003; Lynes et al. 2006; Knepper and Pisitkun 2007). None of these potential sources of urine MT have been studied in more detail. As mentioned previously (c.f. Fig. 6), PT cells in adult rats were stained for MT with heterogeneous intensity, and many cells or whole tubule profiles showed no staining at all. However, our unpublished immunocytochemical study of MT in cryosections of adult rat kidneys detected in the lumen of some MT-rich convoluted PT distinct, droplet-like formations that were strongly positive for MT (Fig. 9). These MT droplets were variable in size (diameter 0.5–5 μm), and seemed to be extruded from the cell apical domain; some droplets were detached (arrowheads), and some were still connected to the cell (arrows) (Fig. 9A, B). A similar MT-positive material in the PT lumen was previously described in rats (Danielson et al. 1982a, b; Tohyama et al. 1988), but the phenomenon has attracted no further attention. The fixation and staining techniques in our (*p*-formaldehyde-lysine-periodate (PLP) fixation in vivo and indirect immunofluorescence staining in frozen tissue sections) and in previous studies (fixation in Bouin's or 10% buffered formalin fixative by immersion and staining by immunoperoxidase method in paraffin sections) were different, thus indicating that the MT-positive material in the PT lumen was not related to specific fixation and/or staining artefacts. Furthermore, in our studies, in the lumen of lower nephron segments, MT droplets were absent (not shown). These data thus indicate that the MT droplets may have been extruded from the MT-rich cells of the cortical PT (c.f., Fig. 6E), and became more fragmented/dissolved during the passage through more distal nephron segments. We thus assume that in intact animals these MT droplets (exovesicles) may form and are released during constitutive remodelling of the cell apical membrane, and may be precursors of some exosomes and source of MT in the urine. The phenomenon, that the MT droplet formation may be associated only with the cells that exhibited a high content of cytoplasmic MT, indicates that the process may be somehow regulated, and possibly induced in order to remove/secrete a part of the over-accumulated MT (as apo-MT, ZnMT, CdMT, or some other complex with toxic metals). This kind of MT secretion may resemble that described in the cell lines of human intestinal enterocytes (Moltedo et al. 2000) and mouse adipocytes (Trayhurn et al. 2000). In the tubule fluid along the nephron, apo-MT may play a protective role as a metal and ROS scavenger.

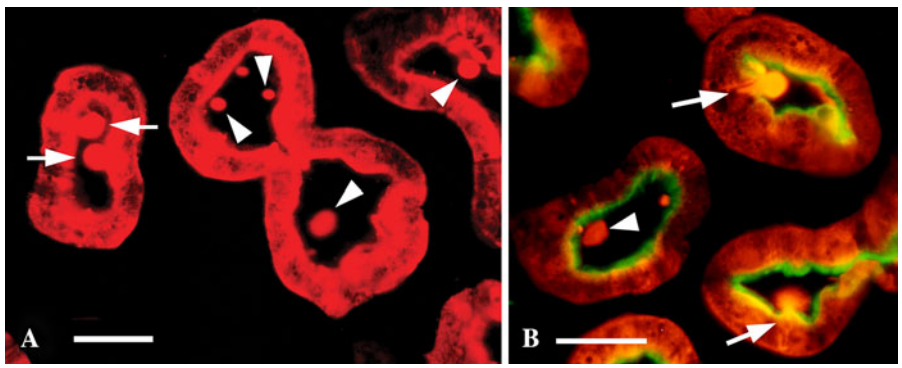

Fig. 9 MT droplets in some PT profiles of the rat kidney cortex. MT-rich droplets appear as free formations in the tubule lumen (**A** and **B**, *arrowheads*) or as formations still connected to the tubule cell apical domain (**A** and **B**, *arrows*). According to the immunocytochemical presentation in (**B**), where the PT were double stained for MT (*red fluorescence*) and megalin (*green fluorescence* in brush-border membrane), the MT droplets may be formed by expulsion (exocytosis) of the MT-rich content from the cells that contained a high amount of MT in their cytoplasm (*arrows*)

Metallothionein in Cd-induced toxicity in male and female reproductive tracts

In adult male rats, acute or chronic treatment with $CdCl_2$ induces toxic effects on reproductive organs, such as a reduction in size and mass of the testes, epididymides and seminal vesicles, and loss of reproductive potency; Cd causes vascular damage and necrosis of seminiferous tubules, and directly interferes with specific secretory functions in the reproductive tract epithelium (Nordberg 1975; Laskey et al. 1984; Herak-Kramberger et al. 2000, and references there in; Siu et al. 2009). The role of MT in Cd-induced damage of the male reproductive organs is, however, questionable. In a few in vitro studies, in an established Leydig cell line treated with Zn or Cd (Shiraishi et al. 1995) and in freshly isolated testicular interstitial, Sertoli and spermatogenic cells from Cd-treated rats (McKenna et al. 1996; Ren et al. 2003a, b), no significant upregulation of MT and its mRNA synthesis was observed. Furthermore, the overexpression of MT-1 in TG mice did not protect from Cd-induced testicular necrosis and reduced fertility (Dalton et al. 1996a). Rather, Cd effects in mice were largely strain-dependent due to genetically determined expression of the Cd transporter ZIP-8, and not due to the MT phenotype (Taylor et al. 1973; Liu et al. 2001; Dalton et al. 2005). In the female reproductive tract, however, Cd treatment in rats resulted in estrogen-like, proliferative effects (Johnson et al. 2003), whereas in rabbits and mice, it caused degenerative changes in various parts of the reproductive tract and reduced pregnancy rate (Dalton et al. 1996a; Massany et al. 2007). The impaired reproduction was present also in Cd-treated TG, MT-1-overexpressing female mice (Dalton et al. 1996a). Therefore, it seems that in both male and female reproductive tracts MT plays no significant role in fighting Cd-induced injury, but this problem needs further testing.

Metallothionein in other Cd toxicity-related diseases in mammalian organs

Long-term exposure to Cd in humans and experimental animals leads to accumulation of the metal in bones, more prominently in females, and accelerates loss of Ca^{2+} from the bones and its excretion in the urine, thus increasing the risk of kidney stones, osteomalacia and osteoporosis (reviewed in: Nomiyama 1980; Järup et al. 1998; Kazantzis 2004; Bhattacharyya 2009; Nordberg 2009). Cd-treatment of rats was shown to upregulate MT-1/MT-2 proteins and mRNA in bone cells (Oda et al. 2001). Other studies have shown that MT plays an important protective, anti-osteoporotic role in Cd intoxication, as confirmed in Cd-treated MT-null mice, in which the bone density strongly decreased due to accelerated release of Ca^{2+} (Habeebu et al. 2000b; reviewed in Bhattacharyya 2009). This contemporary health problem in human society, especially among women, requires highest attention. Furthermore, chronic environmental or experimental exposure to Cd in humans and animals is manifested by anemia and impaired immunity (reviewed in: Nomiyama 1980; Klaassen et al. 2009). In causing these symptoms, Cd can affect the hematopoietic cells directly or their production. The mammalian red blood cells, lymphocytes, and platelets contain a limited amount of Cd-inducible MT (Sagiura and Nakamura 1994; Rahman et al. 2000; Lu et al. 2005); in Cd-treated rats, a parallel accumulation of Cd and MT was observed in hematopoietic cells in bone marrow, thymus and spleen (Yamano et al. 1998). The hematotoxic and immunotoxic effects of Cd were much worse in MT-1/MT-2-null mice than in WT mice, indicating that MT in hematopoietic tissues plays a role similar to that in the liver and kidneys, e.g., protection from oxidative stress (Liu et al. 1999a, b).

As found by Chen et al. (2001), TG mice with overexpression of MT in pancreatic beta-cells exhibit less severe streptozotocin-induced diabetes, and reduced DNA damage. However, epidemiological surveys in humans, and experimental studies in rodents, have shown that patients with type 2 diabetes and streptozotocin-induced diabetic rats develop more severe Cd-induced nephropathy than the non-diabetic patients or animals (reviewed in: Järup et al. 1998; Edwards and Prozialeck 2009). On the other side, treatment of rats with streptozotocin resulted in an increased MT synthesis in the liver and kidneys, which gave a significant protection against acute CdMT-induced nephrotoxicity (Jin et al. 1996).

Recent epidemiological studies in humans showed that Cd-exposed patients with a higher prevalence of kidney dysfunction contain anti-MT antibodies in their blood plasma, and that these antibodies could be

used as a biomarker for severity of this condition (Chen et al. 2006a, c). The anti-MT antibodies in the diabetic plasma could diminish the antioxidative function of MT in blood and other organs, including kidneys, by an unknown mechanism.

MT plays a major protective role in ischemia–reperfusion injury of the heart; the postischemic recovery of functional characteristics in isolated heart from MT-TG animals was increased compared to that from non-TG animals (Kang et al. 1999). Moreover, Cd administration in rats caused an upregulation of the MT content in the heart (Bobillier-Chaumont et al. 2006), and this condition also provided cardioprotection against ischemia–reperfusion injury (Devaux et al. 2009). The studies indicate that upregulation of MT in the heart may be an important protective mechanism against ROS-mediated cell damage in Cd intoxication and ischemia–reperfusion. This, potentially important role of MT in the pathophysiology of ischemic heart disease in humans, has not been sufficiently studied.

A number of epidemiological and experimental data indicate that chronic exposure to Cd in humans and animals can be associated with cancerogenesis, primarily in the lung, but possibly also in the prostate, kidneys, breast, urinary bladder, testis, pancreas, stomach and hematopoietic system (reviewed in: Waalkes 2000; Huff et al. 2007). The cellular and molecular mechanisms of Cd-induced carcinogenesis have been reviewed elsewhere (Waisberg et al. 2003; Liu et al. 2009; Joseph 2009). As already mentioned, mice having a higher content of MT in the lungs exhibited less Cd-induced toxicity and a lower incidence of lung cancer than rats expressing less MT (Kenaga et al. 1996; McKenna et al. 1998), whereas Cd-treated MT-null mice exhibited an increased incidence of hepatic carcinoma in comparison with WT mice (Habeebu et al. 2000a; reviewed in Klaassen et al. 2009). In these cases, sequestering Cd and scavenging ROS during Cd-induced oxidative stress may be the major role of MT, and these functions protect from triggering further events, such as activation of oncogenes, stress-response genes and transcription factors, and inhibition of DNA damage repair, which finally lead to apoptosis (programmed cell death) or uncontrolled cell proliferation (reviewed in: Waisberg et al. 2003; Liu et al. 2009; Joseph 2009). However, various human tumors (not associated with Cd-exposure), particularly their metastases, frequently exhibit over-expression of MT, but the underlying mechanisms for this phenomenon are not known. The upregulation of MT in cancer cells may be a compensatory mechanism in combating oxidative stress, which is usually present in this fast-growing and hypoxic tissue, and which can make the cells apoptosis-resistant (Qu et al. 2006; Somji et al. 2006, and references therein). Yet, in different studies very different findings for the relative number of MT-positive and MT-negative (unstained) cells were reported even in the same types of cancers, and the correlation between the levels of MT expression in cancer tissues and the degree of malignancy or drug and radiation resistance, was also contradictory (Theocharis et al. 2003). For humans, this very important field of medicine should be studied with the innovative experimental approaches.

Acknowledgements The authors thank Mrs. Eva Heršak for technical assistance in performing experiments. This work was supported by grant 022-0222148-2146 from Ministry for Science, Education and Sports, Republic of Croatia (I.S.).

References

Abouhamed M, Gburek J, Liu W et al (2006) Divalent metal transporter 1 in the kidney proximal tubule is expressed in late endosomes/lysosomal membranes: implications for renal handling of protein-metal complexes. Am J Physiol Renal Physiol 290:F1525–F1533

Abouhamed M, Wolff NA, Lee WK et al (2007) Knockdown of endosomal/lysosomal divalent metal transporter 1 by RNA interference prevents cadmium-metallothionein-1 cytotoxicity in renal proximal tubule cells. Am J Physiol Renal Physiol 293:F705–F712

Åkesson A, Berglund M, Schütz A et al (2002) Cadmium exposure in pregnancy and lactation in relation to iron status. Am J Public Health 92:284–287

Andrews GK (2000) Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochem Pharmacol 59:95–104

Andrews GK, Adamson ED, Gedamu L (1984) The ontogeny of expression of murine metallothionein: comparison with the alpha-fetoprotein gene. Dev Biol 103:294–303

Aschner M, West AK (2005) The role of MT in neurological disorders. J Alzheimers Dis 8:139–145

Aschner M, Cherian MG, Klaassen CD et al (1997) Metallothioneins in brain—the role in physiology and pathology. Toxicol Appl Pharmacol 142:229–242

Baker TK, VanVooren HB, Smith WC et al (2003) Involvement of calcium channels in the sexual dimorphism of cadmium-induced hepatotoxicity. Toxicol Lett 137:185–192

Bergendi L, Benes L, Durackova Z et al (1999) Chemistry, physiology and pathology of free radicals. Life Sci 65:1865–1874

Berglund M, Åkesson A, Nermell B et al (1994) Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. Environ Health Perspect 102:1058–1066

Bhattacharyya MH (2009) Cadmium osteotoxicity in experimental animals: mechanisms and relationship to human exposures. Toxicol Appl Pharmacol 238:258–265

Bittel D, Dalton T, Samson SLA et al (1998) The DNA binding activity of metal response element-binding transcriptional factor-1 is activated in vivo and in vitro by Zn, but not by other transition metals. J Biol Chem 273:7127–7133

Blazka ME, Shaikh ZA (1991) Sex differences in hepatic and renal cadmium accumulation and metallothionein induction. Role of estradiol. Biochem Pharmacol 41:775–780

Blazka ME, Nolan CV, Shaikh ZA (1988) Developmental and sex differences in cadmium distribution and metallothionein induction and localization. J Appl Toxicol 8:217–222

Bobillier-Chaumont S, Maupoil V, Berthelot A (2006) Metallothionein induction in the liver, kidney, heart and aorta of cadmium and isoproterenol treated rats. J Appl Toxicol 26:47–55

Bremner I (1987) Involvement of metallothionein in the hepatic metabolism of copper. J Nutr 117:19–29

Bridges CC, Zalups RK (2005) Molecular and ionic mimicry and the transport of toxic metals. Toxicol Appl Pharmacol 204:274–308

Brookes PS, Yoon Y, Robotham JL et al (2004) Calcium, ATP, and ROS: a mitochondrial love-hate triangle. Am J Physiol Cell Physiol 287:C817–C833

Casalino E, Sblano C, Landriscina C (1997) Enzyme activity alteration by cadmium administration to rats: the possibility of iron involvement in lipid peroxidation. Arch Biochem Biophys 346:171–179

Chan HM, Cherian MG (1993) Ontogenic changes in hepatic metallothionein isoforms in prenatal and newborn rats. Biochem Cell Biol 71:133–140

Chan HM, Zhu LF, Zhong R et al (1993) Nephrotoxicity in rats following liver transplantation from cadmium-exposed rats. Toxicol Appl Pharmacol 123:89–96

Chang X, Jin T, Chen L et al (2009) Metallothionein I isoform mRNA expression in peripheral lymphocytes as a biomarker for occupational cadmium exposure. Exp Biol Med 234:666–672

Chen H, Carlson EC, Pellet L et al (2001) Overexpression of metallothionein in pancreatic beta cells reduces streptozotocin-induced DNA damage and diabetes. Diabetes 50:2040–2046

Chen L, Jin T, Huang R et al (2006a) Plasma metallothionein antibody and cadmium-induced renal dysfunction in an occupational population in China. Toxicol Sci 91: 104–112

Chen L, Jin T, Huang B et al (2006b) Critical exposure level of cadmium for elevated urinary metallothionein–an occupational population study in China. Toxicol Appl Pharmacol 215:93–99

Chen L, Lei L, Jin T et al (2006c) Plasma metallothionein antibody, urinary cadmium, and renal dysfunction in a Chinese type 2 diabetic population. Diabetes Care 29:2682–2687

Cherian MG (1979) Metabolism of orally administered cadmium-metallothionein in mice. Environ Health Perspect 28:127–130

Cherian MG (1983) Absorption and tissue distribution of cadmium in mice after chronic feeding with cadmium chloride and cadmium-metallothionein. Bull Environm Contam Toxicol 30:33–36

Cherian MG (1994) The significance of the nuclear and cytoplasmic localization of metallothionein in human liver and tumor cells. Environ Health Perspect 102(Suppl. 3):131–135

Cherian MG, Apostolova MD (2000) Nuclear localization of metallothionein during cell proliferation and differentiation. Cell Molec Biol 46:347–356

Cherian MG, Kang YJ (2006) Metallothionein and liver regeneration. Exp Biol Med 231:138–144

Cherian MG, Goyer RA, Delaquerriere-Richardson L (1976) Cadmium-metallothionein-induced nephropathy. Toxicol Appl Pharmacol 38:399–408

Cherian MG, Goyer RA, Valberg LS (1978) Gastrointestinal absorption and organ distribution of oral cadmium chloride and cadmium-metallothioneine in mice. J Toxicol Environ Health 4:861–868

Childers M, Eckel G, Himmel A et al (2007) A new model of cystic fibrosis pathology: lack of transport of glutathione and its thiocyanate conjugates. Med Hypothes 68: 101–112

Choudhuri S, McKim JM, Klaassen CD (1992) Role of hepatic lysosomes in the degradation of metallothionein. Toxicol Appl Pharmacol 115:64–71

Christensen EI, Verroust PJ, Nielsen R (2009) Receptor-mediated endocytosis in renal proximal tubule. Pflugers Arch Eur J Physiol 458:1039–1048

Cole SPC, Deeley RG (2006) Transport of glutathione and glutathione conjugates by MRP1. Trends Pharmacol Sci 27:438–446

D'Amico G, Bazzi C (2003) Pathophysiology of proteinuria. Kidney Int 63:809–825

Dalton T, Fu K, Enders GC et al (1996a) Analysis of the effects of overexpression of metallothionein-1 in transgenic mice on the reproductive toxicology of cadmium. Environ Health Perspect 104:68–76

Dalton TP, Fu K, Palmiter RD et al (1996b) Transgenic mice that overexpress metallothionein-1 resist dietary zinc deficiency. J Nutr 126:825–833

Dalton TP, Li QW, Bittel D et al (1996c) Oxidative stress activates metal-responsive transcription factor-1 binding activity. Occupancy in vivo of metal response elements in the metallothionein-1 gene promotor. J Biol Chem 271:26233–26241

Dalton TP, He L, Wang B et al (2005) Identification of mouse SLC39A8 as a transporter responsible for cadmium-induced toxicity in the testis. Proc Natl Acad Sci USA 102:3401–3406

Danielson KG, Ohi S, Huang PC (1982a) Immunochemical detection of metallothionein in specific epithelial cells of rat organs. Proc Natl Acad Sci USA 79:2301–2304

Danielson KG, Ohi S, Huang PC (1982b) Immunochemical localization of metallothionein in rat liver and kidney. J Histochem Cytochem 30:1033–1039

Davis SR, Cousins RJ (2000) Metallothionein expression in animals: a physiological perspective on function. J Nutr 130:1085–1088

Day FA, Funk AE, Brady FO (1984) In vivo and ex vivo displacement of zinc from metallothionein by cadmium and by mercury. Chem Biol Interact 50:159–174

Devaux S, Maupoil V, Berthelot A (2009) Effects of cadmium on cardiac metallothionein induction and ischemia-reperfusion injury in rats. Can J Physiol Pharmacol 87:617–623

Dorian C, Klaassen CD (1995) Protection by zinc-metallothionein (ZnMT) against cadmium-metallothionein-induced nephrotoxicity. Fundam Appl Toxicol 26:99–106

Dorian C, Gattone VH II, Klaassen CD (1992) Accumulation and degradation of the protein moiety of cadmium-metallothionein (CdMT) in the mouse kidney. Toxicol Appl Pharmacol 117:242–248

Dorian C, Gattone VH II, Klaassen CD (1995) Discrepancy between the nephrotoxic potencies of cadmium-metallothionein and cadmium chloride and the renal concentration of cadmium in the proximal convoluted tubules. Toxicol Appl Pharmacol 130:161–168

Dubey RK, Jackson EK (2001) Estrogen-induced cardiorenal protection: potential cellular, biochemical, and molecular mechanisms. Am J Physiol Renal Physiol 280:F365–F388

Dudley RE, Gammal LM, Klaassen CD (1985) Cadmium-induced hepatic and renal injury in chronically exposed rats: likely role of hepatic cadmium-metallothionein in nephrotoxicity. Toxicol Appl Pharmacol 77:414–426

Ebadi M, Iversen PL, Hao R et al (1995) Expression and regulation of brain metallothionein. Neurochem Int 27: 1–22

Edwards JR, Prozialeck WC (2009) Cadmium, diabetes and chronic kidney disease. Toxicol Appl Pharmacol 238:289–293

Eisenhans B, Strugala GJ, Schafer SG (1997) Small-intestinal absorption of cadmium and the significance of mucosal metallothionein. Human Exp Toxicol 16:429–434

Erfurt C, Roussa E, Thevenod F (2003) Apoptosis by Cd^{2+} or CdMT in proximal tubule cells: different uptake routes and permissive role of endo/lysosomal CdMT uptake. Am J Physiol Cell Physiol 285:C1367–C1376

Feldman SL, Failla ML, Cousins RJ (1978) Degradation of rat liver metallothioneins in vitro. Biochim Biophys Acta 544:638–646

Folch J, Ortega A, Cabre A et al (1998) Urinary levels of metallothioneins and metals in subjects from a semiindustrialized area in Tarragona province of Spain. Biol Trace Elem Res 63:113–121

Freisinger E (2008) Plant MTs—long neglected members of the metallothionein superfamily. Dalton Transact 47:6663–6675

Fujishiro H, Okugaki S, Kubota K et al (2009) The role of ZIP-8 down-regulation in cadmium-resistant metallothionein-null cells. J Appl Toxicol 29:367–373

Garrett SH, Sens MA, Todd JH et al (1999) Expression of MT-3 protein in the human kidney. Toxicol Lett 105:207–214

Garrett SH, Phillips V, Somji S et al (2002) Transient induction of metallothionein isoform 3 (MT-3), c-fos, c-jun, and c-myc in human proximal tubule cells exposed to cadmium. Toxicol Lett 126:69–80

Ghio AJ, Piantadosi CA, Wang XC et al (2005) Divalent metal transporter-1 decreases metal-related injury in the lung. Am J Physiol Lung Cell Mol Physiol 289:L460–L467

Goering PL, Klaasen CD (1984a) Resistance to cadmium-induced hepatotoxicity in immature rats. Toxicol Appl Pharmacol 74:321–329

Goering PL, Klaassen CD (1984b) Tolerance to cadmium-induced toxicity depends on presynthesized metallothionein in liver. J Toxicol Environ Health 14:803–812

Goering PL, Klaassen CD (1984c) Zinc-induced tolerance to cadmium hepatotoxicity. Toxicol Appl Pharmacol 74:299–307

Gunes C, Heuchel R, Georgiev O et al (1998) Embryonic lethality and liver degeneration in mice lacking the metal-responsive transcriptional activator MTF-1. EMBO J 17:2846–2854

Gunshin H, Mackenzie B, Berger UV et al (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388:482–488

Habeebu SS, Liu J, Liu Y, Klaassen CD (2000a) Metallothionein-null mice are more sensitive than wild-type mice to liver injury induced by repeated exposure to cadmium. Toxicol Sci 55:223–232

Habeebu SS, Liu J, Liu YP et al (2000b) Metallothionein-null mice are more susceptible than wild-type mice to chronic $CdCl_2$-induced bone injury. Toxicol Sci 56:211–219

Hamer DH (1986) Metallothionein. Ann Rev Biochem 55: 913–951

Hao Q, Hong S-H, Maret W (2007) Lipid raft-dependent endocytosis of metallothionein in HepG2 cells. J Cell Physiol 210:428–435

Haq F, Mahoney M, Koropatnick J (2003) Signalling events for metallothionein induction. Mutat Res 533:211–226

Hart BA, Voss GW, Willean CL (1989) Pulmonary tolerance to cadmium following cadmium aerosol pretreatment. Toxicol Appl Pharmacol 101:447–460

Hart BA, Gong Q, Eneman JD et al (1995) In vivo expression of metallothionein in rat alveolar macrophages and type II epithelial cells following repeated cadmium aerosol exposure. Toxicol Appl Pharmacol 133:82–90

Hart BA, Potts RJ, Watkin RD (2001) Cadmium adaptation in the lung–a double-edged sword. Toxicology 160(1–3): 65–70

Herak-Kramberger CM, Sabolic I (2001) The integrity of renal cortical brush-border and basolateral membrane vesicles is damaged in vitro by nephrotoxic heavy metals. Toxicology 156:139–147

Herak-Kramberger CM, Brown D, Sabolic I (1998) Cadmium inhibits vacuolar H^+-ATPase and endocytosis in rat kidney cortex. Kidney Int 53:1713–1726

Herak-Kramberger CM, Sabolic I, Blanusa M et al (2000) Cadmium inhibits vacuolar H^+-ATPase-mediated acidification in the rat epididymis. Biol Reprod 63:599–606

Heuchel R, Radtke F, Georgiev O et al (1994) The transcription factor MTF-1 is essential for basal and heavy metal-induced metallothionein gene expression. EMBO J 13:2870–2875

Hoey JG, Garrett SH, Sens MA et al (1997) Expression of MT-3 mRNA in human kidney, proximal tubule cell cultures, and renal cell carcinoma. Toxicol Lett 92:149–160

Hozumi I, Suzuki JS, Kanazawa H et al (2008) Metallothionein-3 is expressed in the brain and various peripheral organs of the rat. Neurosci Lett 438:54–58

Huff J, Lunn RM, Waalkes MP et al (2007) Cadmium-induced cancers in animals and in humans. Int J Occup Environ Health 13:202–212

Hunziker PE, Kaur P, Wan M et al (1995) Primary structures of 7 metallothionenis from rabbit tissue. Biochem J 306:265–270

Hutton M (1983) Sources of cadmium in the environment. Ecotoxicol Environ Safety 7:9–24

Iszard MB, Liu J, Liu YP et al (1995) Characterization of metallothionein-I-transgenic mice. Toxicol Appl Pharmacol 133:305–312

Itoh S, Kim HW, Nakagawa O et al (2008) Novel role of antioxidant-1 (Atox1) as a copper-dependent transcription factor involved in cell proliferation. J Biol Chem 283:9157–9167

Jacob C, Maret W, Vallee BL (1998) Control of zinc transfer between thionein, metallothionein, and zinc proteins. Proc Natl Acad Sci USA 95:3489–3494

Jacquilett G, Barbier O, Cougnon M et al (2006) Zinc protects renal function during cadmium intoxication in the rat. Am J Physiol Renal Physiol 290:F127–F137

Järup L, Akesson A (2009) Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol 238:201–208

Järup L, Berglund M, Elinder CG et al (1998) Health effects of cadmium exposure—a review of the literature and a risk estimate. Scand J Work Environ Health 24(Suppl. 1):1–52

Jeong SH, Habeebu SSM, Klaassen CD (2000) Cadmium decreases gap junctional intercellular communications in mouse liver. Toxicol Sci 57:156–166

Jiang LJ, Maret W, Vallee BL (1998a) The ATP-metallothionein complex. Proc Natl Acad Sci USA 95:9146–9149

Jiang LJ, Maret W, Vallee BL (1998b) The glutathione redox couple modulates zinc transfer from metallothionein to zinc-depleted sorbitol dehydrogenase. Proc Natl Acad Sci USA 95:3483–3488

Jin T, Nordberg GF, Nordberg M (1987) Resistance to acute nephrotoxicity induced by cadmium-metallothionein dependence on pretreatment with cadmium chloride. Pharmacol Toxicol 61:89–93

Jin TY, Nordberg G, Sehlin J, Vesterberg O (1996) Protection against cadmium-metallothionein nephrotoxicity in streptozotocin-induced diabetic rats—role of increased metallo-thionein synthesis induced by streptozotocin. Toxicology 106:55–63

Johnson MD, Kenney N, Stoica A et al (2003) Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. Nature Med 9:1081–1084

Joseph P (2009) Mechanisms of cadmium carcinogenesis. Toxicol Appl Pharmacol 238:272–279

Kägi JHR, Vallee BL (1960) Metallothionein: a cadmium- and zinc-containing protein from equine renal cortex. J Biol Chem 235:3460–3465

Kang YJ (2006) Metallothionein redox cycle. Exp Biol Med 231:1459–1467

Kang YJ, Li GQ, Saari JT (1999) Metallothionein inhibits ischemia-reperfusion injury in mouse heart. Am J Physiol Heart Circ Physiol 45:H993–H997

Kazantzis G (2004) Cadmium, osteoporosis and calcium metabolism. Biometals 17:493–498

Kenaga C, Cherian MG, Cox C et al (1996) Metallothionein induction and pulmonary responses to inhaled cadmium chloride in rats and mice. Fundam Appl Toxicol 30: 204–212

Kershaw WC, Klaassen (1992) Degradation and metal composition of hepatic isometallothioneins in rats. Toxicol Appl Pharmacol 112:24–31

Khan I, Ahmad S, Thomas N (1998) Differential translation of the alpha-1 isoforms of L-type calcium channel in rat brain and other tissues. Biochem Molec Biol Int 45:895–904

Kher A, Meldrum KK, Wang MJ et al (2005) Cellular and molecular mechanisms of sex differences in renal ischemia-reperfusion injury. Cardiovasc Res 67:594–603

Kim D, Garrett SH, Sens MA et al (2002) Metallothionein isoform 3 and proximal tubule vectorial active transport. Kidney Int 61:464–472

Kimura T, Itoh N, Min KS et al (1998) Tissue accumulation of cadmium following oral administration to metallothionein-null mice. Toxicol Lett 99:85–90

Klaassen CD, Lehman-McKeeman LD (1989) Regulation of the isoforms of metallothionein. Biol Trace Elem Res 21:119–129

Klaassen CD, Liu J (1997) Role of metallothionein in cadmium-induced hepatotoxicity and nephrotoxicity. Drug Metab Rev 29:79–102

Klaassen CD, Choudhuri S, McKim JM Jr et al (1994) In vitro and in vivo studies on degradation of metallothionein. Environ Health Perspect 102(Suppl 3):141–146

Klaassen CD, Liu J, Choudhuri S (1999) Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu Rev Pharmacol Toxicol 39:267–294

Klaassen CD, Liu J, Diwan BA (2009) Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 238:215–220

Klassen RB, Crenshaw K, Kozyraki R et al (2004) Megalin mediates renal uptake of heavy metal metallothionein complexes. Am J Physiol Renal Physiol 287:F393–F403

Knepper MA, Pisitkun T (2007) Exosomes in urine: who would have thought.? Kidney Int 72:1043–1045

Koh AS, Simmons-Willis TA, Pritchard JB et al (2002) Identification of a mechanism by which the methylmercury antidotes N-acetylcysteine and dimercaptopropanesulfonate enhance urinary metal excretion: Transport by the renal organic anion transporter-1. Mol Pharmacol 62:921–926

Kourie JI (1998) Interaction of reactive oxygen species with ion transport mechanisms. Am J Physiol Cell Physiol 275:C1–C24

Kuester RK, Waalkes MP, Goering PL et al (2002) Differential hepatotoxicity induced by cadmium in Fischer 344 and Sprague-Dawley rats. Toxicol Sci 65:151–159

LaRochelle O, Gagne V, Charron J et al (2001) Phosphorylation is involved in the activation of metal-regulatory transcription factor 1 in response to metal ions. J Biol Chem 276:41879–41888

Lash LH, Pull DA, Xu F et al (2007) Role of rat organic anion transporter 3 (Oat3) in the renal basolateral transport of glutathione. Chem Biol Interact 170:124–134

Laskey ', Rehnberg GL, Laws SC et al (1984) Reproductive effects of low acute doses of cadmium chloride in adult male rats. Toxicol Appl Pharmacol 73:250–255

Lee WK, Bork U, Gholamrezaei F, Thevenod F (2005) Cd^{2+}-induced cytochrome c release in apoptotic proximal tubule cells: role of mitochondrial permeability transition pore and Ca^{2+} uniporter. Am J Physiol Renal Physiol 288:F27–F39

Lehman LD, Klaassen CD (1986) Dosage-dependent disposition of cadmium administered orally to rats. Toxicol Appl Pharmacol 84:159–167

Levadoux M, Mahon C, Beattie JH et al (1999) Nuclear import of metallothionein requires its mRNA to be associated with the perinuclear cytoskeleton. J Biol Chem 274:34961–34966

Liu J, Klaassen CD (1996) Absorption and distribution of cadmium in metallothionein-I transgenic mice. Fund Appl Toxicol 29:294–300

Liu J, Liu Y, Klaassen CD (1994) Nephrotoxicity of $CdCl_2$ and Cd-metallothionein in cultured rat kidney proximal tubules and LLC-PK1 cells. Toxicol Appl Pharmacol 128:264–270

Liu YP, Liu J, Iszard MB et al (1995) Transgenic mice that overexpress metallothionein-1 are protected from cadmium lethality and hepatotoxicity. Toxicol Appl Pharmacol 135:222–228

Liu J, Liu Y, Michalska AE et al (1996a) Distribution and retention of cadmium in metallothionein I and II null mice. Toxicol Appl Pharmacol 136:260–268

Liu Y, Liu J, Palmiter R et al (1996b) Metallothionein-I-transgenic mice are not protected from acute cadmium-metallothionein-induced nephrotoxicity. Toxicol Appl Pharmacol 137:307–315

Liu J, Liu Y, Habeebu SS et al (1998) Susceptibility of MT-null mice to chronic CdCl2-induced nephrotoxicity indicates that renal injury is not mediated by the CdMT complex. Toxicol Sci 46:197–203

Liu J, Liu YP, Habeebu SS et al (1999a) Metallothionein-null mice are highly susceptible to the hematotoxic and immunotoxic effects of chronic $CdCl_2$ exposure. Toxicol Appl Pharmacol 159:98–108

Liu YP, Liu J, Habeebu SM et al (1999b) Metallothionein protects against the nephrotoxicity produced by chronic CdMT exposure. Toxicol Sci 50:221–227

Liu J, Corton C, Dix DJ et al (2001a) Genetic background but not metallothionein phenotype dictates sensitivity to cadmium-induced testicular injury in mice. Toxicol Appl Pharmacol 176:1–9

Liu YP, Liu J, Klaassen CD (2001b) Metallothionein-null and wild-type mice show similar cadmium absorption and tissue distribution following oral cadmium administration. Toxicol Appl Pharmacol 175:253–259

Liu J, Cheng ML, Yang Q et al (2007) Blood metallothionein transcript as a biomarker for metal sensitivity: low blood metallothionein transcripts in arsenicosis patients from Guizhou, China. Environ Health Perspect 115:1101–1106

Liu J, Qu W, Kadiiska MB (2009) Role of oxidative stress in cadmium toxicity and carcinogenicity. Toxicol Appl Pharmacol 238:209–214

Lu H, Jin TY, Nordberg G et al (2005) Metallothionein gene expression in peripheral lymphocytes and renal dysfunction in a population environmentally exposed to cadmium. Toxicol Appl Pharmacol 206:150–156

Lynes MA, Zaffuto K, Unfricht DW et al (2006) The physiological roles of extracellular metallothionein. Exp Biol Med 231:1548–1554

Maret W (2003) Cellular zinc and redox states converge in the metallothionein/thionein pair. J Nutr 133:1460S–1462S

Marshansky V, Bourgoin S, Londono I et al (1997) Receptor-mediated endocytosis in kidney proximal tubules: recent advances and hypothesis. Electrophoresis 18: 2661–2676

Massany P, Lukac N, Uhrin V et al (2007) Female reproductive toxicology of cadmium. Acta Biol Hungar 58:287–299

Masters BA, Kelly E, Quaife CJ et al (1994) Targeted disruption of metallothionein I and II genes increase sensitivity to cadmium. Proc Natl Acad Sci USA 91:584–588

McKenna IM, Bare RM, Waalkes MP (1996) Metallothionein gene expression in testicular interstitial cells and liver of rats treated with cadmium. Toxicology 107:121–130

McKenna IM, Gordon T, Chen LC et al (1998) Expression of metallothionein protein in the lungs of Wistar rats and C57 and DBA mice exposed to cadmium oxide fumes. Toxicol Appl Pharmacol 153:169–178

McKim JM Jr, Choudhuri S, Klaassen CD (1992) In vitro degradation of apo-, zinc-, and cadmium-metallothionein by cathepsin B, C, and D. Toxicol Appl Pharmacol 116:117–124

Mendez-Armenta M, Rios C (2007) Cadmium neurotoxicity. Environ Toxicol Pharmacol 23:350–358

Mesna OJ, Wilhelmsen TW, Andersen RA (2000) Correlations between cadmium treatment, oxygen uptake and metallothionein response in liver and kidney from two mice strains. Comp Biochem Physiol B 125:21–27

Mididoddi S, McGuirt JP, Sens MA et al (1996) Isoform-specific expression of metallothionein mRNA in the developing and adult human kidney. Toxicol Lett 85: 17–27

Miles AT, Hawksworth GM, Beattie JH et al (2000) Induction, regulation, degradation, and biological significance of mammalian metallothioneins. Crit Rev Biochem Mol Biol 35:35–70

Milnerwicz H, Jablonowska M, Bizon A (2009) Change of zinc, copper, and metallothionein concentrations and the copper-zinc superoxide dismutase activity in patients with pancreatitis. Pancreas 38:681–688

Min KS, Hatta A, Onosaka S et al (1987) Protective role of renal metallothionein against Cd nephrotoxicity in rats. Toxicol Appl Pharmacol 88:294–301

Misra RR, Crance KA, Bare RM et al (1997) Lack of correlation between the inducibility of metallothionein mRNA and metallothionein protein in cadmium-exposed rodents. Toxicology 117:99–109

Moffatt P, Denizeau F (1997) Metallothionein in physiological and pathophysiological processes. Drug Metab Rev 29:261–307

Moffatt P, Seguin C (1998) Expression of the gene encoding metallothionein-3 in organs of the reproductive system. DNA Cell Biol 17:501–510

Moltedo O, Verde C, Capasso A et al (2000) Zinc transport and metallothionein secretion in the intestinal human cell line Caco-2. J Biol Chem 275:31819–31825

Nagano T, Itoh N, Ebisutani C et al (2000) The transport mechanism of metallothionein is different from that of classical NLS-bearing protein. J Cell Physiol 185:440–446

Nickel W (2003) The mystery of nonclassical protein secretion. A current view on cargo proteins and potential export routes. Eur J Biochem 270:2109–2119

Nielson KB, Atkin CL, Winge DR (1985) Distinct metal-binding configurations in metallothionein. J Biol Chem 260:5342–5350

Nishimura H, Nishimura N, Tohyama C (1989a) Immunohistochemical localization of metallothionein in developing rat tissues. J Histochem Cytochem 37:715–722

Nishimura N, Nishimura H, Tohyama C (1989b) Localization of metallothionein in female reproductive organs of rat and Guinea pig. J Histochem Cytochem 37:1601–1607

Nomiyama K (1980) Recent progress and perspectives in cadmium health effects studies. Sci Total Environ 14:199–232

Nomiyama K, Nomiyama H, Kameda N (1998) Plasma cadmium-metallothionein, a biological exposure for cadmium-induced renal dysfunction, based on the mechanism of its action. Toxicology 129:157–168

Nordberg GF (1975) Effects of long-term cadmium exposure on the seminal vesicles of mice. J Reprod Fert 45:165–167

Nordberg M (1998) Metallothionenis: historical review and state of knowledge. Talanta 46:243–254

Nordberg GF (2009) Historical perspectives on cadmium toxicology. Toxicol Appl Pharmacol 238:192–200

Nordberg M, Nordberg GF (2000) Toxicological aspects of metallothionein. Cell Mol Biol 46:451–463

Nordberg M, Nordberg GF (2009) Metallothioneins: historical development and overview. Met Ions Life Sci 5:1–29

Nordberg GF, Garvey JS, Chang CC (1982) Metallothionein in plasma and urine of cadmium workers. Environ Res 28:179–182

Oda N, Sogawa CA, Sogawa N et al (2001) Metallothionein expression and localization in rat bone tissue after cadmium injection. Toxicol Lett 123:143–150

Ogra Y, Suzuki KT (2000) Nuclear trafficking of metallothionein. Possible mechanisms and current knowledge. Cell Mol Biol 46:357–365

Oh SH, Deagen JT, Whanger PD et al (1978) Biological function of metallothionein. V. Its induction in rats by various stresses. Am J Physiol 234:E282–E285

Okamoto T, Jeong SY, Takahashi Y et al (2001) Expression of the alpha 1D subunit of the L-type voltage gated calcium channel in human liver. Int J Mol Med 8:413–416

Olsson IM, Bensryd I, Lundh T et al (2002) Cadmium in blood and urine—impact of sex, age, dietary intake, iron status, and former smoking—association of renal effects. Environ Health Perspect 110:1185–1190

Omarova A, Phillips CJC (2007) A meta-analysis of literature data relating to the relationship between cadmium intake and toxicity indicators in humans. Environ Res 103:432–440

Onosaka S, Cherian MG (1981) Induced synthesis of metallothionein in various tissues of rats in response to metals. I. Repeated cadmium injection. Toxicology 22:91–101

Onosaka S, Min KS, Fujita Y et al (1988) High concentration of pancreatic metallothionein in normal mice. Toxicology 50:27–35

Padanilam BJ (2003) Cell death induced by acute renal injury: a perspective on the contributions of apoptosis and necrosis. Am J Physiol Renal Physiol 284:F608–F627

Palmiter RD (1987) Molecular biology of metallothionein gene expression. Exp Suppl 52:63–80

Palmiter RD (1994) Regulation of metallothionein genes by heavy metals appears to be mediated by a zinc-sensitive inhibitor that interacts with a constitutively active transcription factor, MTF-1. Proc Natl Acad Sci USA 91:1219–1223

Palmiter RD (1998) The elusive function of metallothioneins. Proc Natl Acad Sci USA 95:8428–8430

Palmiter RD, Findley SD, Whitmore TE et al (1992) MT-III, a brain-specific member of the metallothionein gene family. Proc Natl Acad Sci USA 89:6333–6337

Palumaa P, Eriste E, Njunkova O et al (2002) Brain-specific metallothionein-3 has higher metal-binding capacity than ubiquitous metallothioneins and binds metals noncooperatively. Biochemistry 41:6158–6163

Palumaa P, Eriste E, Kruusel K et al (2003) Metal binding to brain-specific metallothionein-3 studied by electrospray ionization mass spectrometry. Cell Mol Biol 49: 763–768

Pattanaik A, Shaw CF III, Petering DH et al (1994) Basal metallothionein in tumors: widespread presence of apoprotein. J Inorg Biochem 54:91–105

Perkins DJ (1961) Studies on the interaction of zinc, cadmium and mercury ions with native and chemically modified human serum albumin. Biochem J 80:668–672

Petering DH, Zhu J, Krezoski S et al (2006) Apo-metallothionein emerging as a major player in the cellular activities of metallothionein. Exp Biol Med 231:1528–1534

Petering DH, Krezoski S, Tabatabai NM (2009) Metallothionein toxicology: metal ion trafficking and cellular protection. Met Ions Life Sci 5:353–397

Pham TND, Segui JA, Fortin C et al (2004) Cadmium uptake in rat hepatocytes in relation to speciation and to complexation with metallothionein and albumin. J Cell Physiol 201:320–330

Powel SR (2000) The antioxidant properties of zinc. J Nutr 130:1447S–1454S

Prozialeck WC (2000) Evidence that E-cadherin may be a target for cadmium toxicity in epithelial cells. Toxicol Appl Pharmacol 164:231–249

Prozialeck WC, Wellington DR, Lamar PC (1993) Comparison of the cytotoxic effects of cadmium chloride and cadmium-metallothionein in LLC-PK1 cells. Life Sci 53:PL337–PL342

Prozialeck WC, Lamar PC, Lynch SA (2003) Cadmium alters the localization of N-cadherin, E-cadherin, and beta-catenin in the proximal tubule epithelium. Toxicol Appl Pharmacol 189:180–195

Qu W, Fuquay R, Sakurai T et al (2006) Acquisition of apoptotic resistance in cadmium-induced malignant transformation: specific perturbation of JNK signal transduction pathway and associated metallothionein overexpression. Mol Carcinog 45:561–571

Quaife CJ, Findley SD, Erickson JC et al (1994) Induction of a new metallothionein isoform (MT-IV) occurs during differentiation of stratified squamous epithelia. Biochemistry 33:7250–7259

Rahman MT, Vandingenen A, DeLey M (2000) Metallothionein biosynthesis in human RBC precursors. Cell Physiol Biochem 10:237–242

Rana SVS (2008) Metals and apoptosis: recent developments. J Trace Elem Med Biol 22:262–284

Regnier A, Dannhoffer L, Blouquit-Laye S et al (2008) Expression of cystic fibrosis transmembrane conductance regulator in the human distal lung. Human Pathol 39:368–376

Ren XY, Zhou Y, Zhang HP et al (2003a) Metallothionein gene expression under different time in testicular Sertoli and spermatogenic cells of rats treated with cadmium. Reprod Toxicol 17:219–227

Ren XY, Zhou Y, Zhang JP et al (2003b) Expression of metallothionein gene at different time in testicular interstitial cells and liver of rats treated with cadmium. World J Gastroenterol 9:1554–1558

Roels H, Lauwerys R, Buchet JP et al (1983) Significance of urinary metallothionein in workers exposed to cadmium. Int Arch Occup Environ Health 52:159–166

Romero-Isart N, Vašak M (2002) Advances in the structure and chemistry of metallothioneins. J Inorg Biochem 88:388–396

Sabolic I (2006) Common mechanisms in nephropathy induced by toxic metals. Nephron Physiol 104:107–114

Sabolic I, Herak-Kramberger CM, Brown D (2001) Subchronic cadmium treatment affects the abundance and arrangement of cytoskeletal proteins in rat renal proximal tubule cells. Toxicology 165:205–216

Sabolic I, Ljubojevic M, Herak-Kramberger CM et al (2002) Cd-MT causes endocytosis of brush-border transporters in rat renal proximal tubules. Am J Physiol Renal Physiol 283:F1389–F1402

Sabolic I, Herak-Kramberger CM, Antolovic R et al (2006) Loss of basolateral invaginations in proximal tubules of cadmium-intoxicated rats is independent of microtubules and clathrin. Toxicology 218:149–163

Sagiura T, Nakamura H (1994) Metallothionein in platelets. Int Arch Allerg Immunol 103:341–348

Samson SLA, Gedamu L (1998) Molecular analyses of metallothionein gene regulation. Prog Nucleic Acid Res Mol Biol 59:257–288

Shaikh ZA, Hirayama K (1979) Metallothionein in the extracellular fluids as an index of cadmium toxicity. Environ Health Perspect 28:267–271

Shaikh ZA, Jordan SA, Tewari PC (1993) Cadmium disposition and metallothionein induction in mice—strain-dependent, sex-dependent, age-dependent and dose-dependent differences. Toxicology 80:51–70

Shaikh ZA, Vu TT, Zaman K (1999) Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. Toxicol Appl Pharmacol 154:256–263

Shimada A, Yanagida M, Umemura T (1997) An immunohistochemical study on the tissue-specific localization of metallothionein in dogs. J Comp Pathol 116:1–11

Shiraishi N, Hochadel JF, Coogan TP et al (1995) Sensitivity to cadmium-induced genotoxicity in rat testicular cells is associated with minimal expression of the metallothionein gene. Toxicol Appl Pharmacol 130:229–236

Simons TJB (1991) Intracellular free zinc and zinc buffering in human red blood cells. J Membr Biol 123:63–71

Siu ER, Mruk DD, Porto CS et al (2009) Cadmium-induced testicular injury. Toxicol Appl Pharmacol 238:240–249

Smirnova IV, Bittel DC, Ravindra R et al (2000) Zinc and cadmium can promote rapid nuclear translocation of metal response element-binding transcription factor-1. J Biol Chem 275:9377–9384

Smith CP, Thevenod F (2009) Iron transport and the kidney. Biochim Biophys Acta 1790:724–730

Solaiman D, Jonah MM, Miyazaki W et al (2001) Increased metallothionein in mouse liver, kidneys, and duodenum during lactation. Toxicol Sci 60:184–192

Somji S, Garrett SH, Sens MA et al (2004) Expression of metallothionein isoform 3 (MT-3) determines the choice between apoptotic or necrotic cell death in Cd^{2+}-exposed human proximal tubule cells. Toxicol Sci 80:358–366

Somji S, Zhou XD, Garrett SH et al (2006) Urothelial cells malignantly transformed by exposure to cadmium (Cd^{2+}) and arsenite (As^{3+}) have increased resistance to Cd^{2+} and As^{3+}-induced cell death. Toxicol Sci 94:293–301

Souza V, Bucio L, Gutierrez-Ruiz MC (1997) Cadmium uptake by a human hepatic cell line (WRL-68 cells). Toxicology 120:215–220

Stillman MJ (1995) Metallothioneins. Coordin Chem Rev 144:461–511

Sugihira N, Tohyama C, Murakami M et al (1986) Significance of increase in urinary metallothionein of rats repeatedly exposed to cadmium. Toxicology 41:1–9

Suhy DA, Simon KD, Linzer DIH et al (1999) Metallothionein is part of a zinc-scavenging mechanism for cell survival under conditions of extreme zinc deprivation. J Biol Chem 274:9183–9192

Susin SA, Zamzami N, Kroemer G (1998) Mitochondria as regulators of apoptosis: doubt no more. Biochim Biophys Acta 1366:151–165

Szczurek EI, Bjornsson CS, Taylor CG (2001) Dietary zinc deficiency and repletion modulate metallothionein immunolocalization and concentration in small intestine and liver of rats. J Nutr 131:2132–2138

Szczurek EI, Bjornsson CS, Noto AD et al (2009) Renal metallothionein responds rapidly and site specifically to zinc repletion in growing rats. J Trace Elem Med Biol 23:176–182

Takahashi Y, Ogra Y, Suzuki KT (2005) Nuclear trafficking of metallothionein requires oxidation of a cytosolic partner. J Cell Physiol 202:563–569

Takano H, Inoue K, Yanagisawa R et al (2004) Protective role of metallothionein in acute lung injury induced by bacterial endotoxin. Thorax 59:1057–1072

Tang W, Shaikh ZA (2001) Renal cortical mitochondrial dysfunction upon cadmium metallothionein administration to Sprague-Dawley rats. J Toxicol Environ Health A 63:221–235

Tapia L, Gonzales-Aguero M, Cisternas MF et al (2004) Metallothionein is crucial for safe intracellular copper storage and cell survival at normal and supra-physiological exposure levels. Biochem J 378:617–624

Taylor BA, Heiniger HJ, Meier H (1973) Genetic analysis of resistance to cadmium-induced testicular damage in mice. Proc Soc Exp Biol Med 143:629–633

Theocharis SE, Margeli AP, Koutselinis A (2003) Metallothionein: a multifunctional protein from toxicity to cancer. Int J Biol Markers 18:162–169

Thevenod F (2003) Nephrotoxicity and the proximal tubule. Insights from cadmium. Nephron Physiol 93:87–93
Thevenod F (2009) Cadmium and signalling cascades: to be or not to be. Toxicol Appl Pharmacol 238:221–239
Thevenod F (2010) Catch me if you can! Novel aspects of cadmium transport in mammalian cells. Biometals. doi: 10.1007/s10534-010-9309-1
Thijssen S, Maringwa J, Faes C et al (2007) Chronic exposure of mice to environmentally relevant, low doses of cadmium leads to early renal damage, not predicted by blood or urine cadmium levels. Toxicology 229:145–156
Tohyama C, Shaikh ZA (1981) Metallothionein in plasma and urine of cadmium-exposed rats determined by a single-antibody radioimmunoassay. Fundam Appl Toxicol 1:1–7
Tohyama C, Shaikh ZA, Ellis KJ et al (1981a) Metallothionein excretion in urine upon cadmium exposure: its relationship with liver and kidney cadmium. Toxicology 22:181–191
Tohyama C, Shaikh ZA, Nogawa K et al (1981b) Elevated urinary excretion of metallothionein due to environmental cadmium exposure. Toxicology 20:289–297
Tohyama C, Nishimura H, Nishimura N (1988) Immunohistochemical localization of metallothionein in the liver and kidney of cadmium- or zinc-treated rats. Acta Histochem Cytochem 21:91–102
Torky ARW, Stehfest E, Viehweger K et al (2005) Immunohistochemical detection of MRPs in human lung cells in culture. Toxicology 207:437–450
Trayhurn P, Duncan JS, Wood AM et al (2000) Metallothionein expression and secretion in white adipose tissue. Am J Physiol Regul Integr Comp Physiol 279:R2329–R2335
Turrens JF (2003) Mitochondrial formation of reactive oxygen species. J Physiol 552:335–344
Vahter M, Akesson A, Liden C et al (2007) Gender differences in the disposition and toxicity of metals. Environ Res 104:85–95
Vallee BL, Ulmer DD (1972) Biochemical effects of mercury, cadmium, and lead. Ann Rev Biochem 41:91–128
Vašak M, Hasler DW (2000) Metallothioneins: new functional and structural insights. Curr Opin Chem Biol 4:177–183
Vasconcetos MH, Tam SC, Hesketh JE et al (2002) Metal- and tissue-dependent relationship between metallothionein mRNA and protein. Toxicol Appl Pharmacol 182:91–97
Verroust PJ, Kozyraki R (2001) The roles of cubilin and megalin, two multiligand receptors, in proximal tubule function: possible implication in the progression of renal disease. Curr Opin Nephrol Hypertens 10:33–38
Waalkes MP (2000) Cadmium carcinogenesis in review. J Inorg Biochem 79:241–244
Waalkes MP, Klaassen CD (1985) Concentration of metallothionein in major organs of rats after administration of various metals. Fundam Appl Toxicol 5:473–477
Waalkes MP, Harvey MJ, Klaassen CD (1984) Relative in vitro affinity of hepatic metallothionein for metals. Toxicol Lett 20:33–39
Waisberg M, Joseph P, Hale B et al (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192:95–117
Wang Y, Fang J, Leonard SS et al (2004a) Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radic Biol Med 36:1434–1443
Wang Y, Wimmer U, Lichtlen P et al (2004b) Metal-responsive transcription factor-1 (MTF-1) is essential for embryonic liver development and heavy metal detoxification in the adult liver. FASEB J 18:1071–1079
Watkins SR, Hodge RM, Cowman DC et al (1977) Cadmium-binding serum proteins. Biochim Biophys Res Commun 74:1408–1410
Webb M (1986) Role of metallothionein in cadmium metabolism. In: Foulkes EC (ed) Handbook of experimental pharmacology, vol 80. Springer, Berlin, pp 281–337
Wesselkamper SC, McDowel SA, Medvedovic M et al (2006) The role of metallothionein in the pathogenesis of acute lung injury. Am J Resp Cell Mol Biol 34:73–82
Wilhelmsen TW, Olsvik PA, Hansen BH et al (2002) Evidence for oligomerization of metallothioneins in their functional state. J Chromatogr A 979:249–254
Wimmer U, Wang Y, Georgiev O et al (2005) Two major branches of anti-cadmium defense in the mouse: MTF-1/metallothioneins and glutathione. Nucl Acid Res 33:5715–5727
Winge DR, Miklossy KA (1982) Domain nature of metallothionein. J Biol Chem 257:3471–3476
Wlostowski T (1993) Involvement of metallothionein and copper in cell proliferation. Biometals 6:71–76
Wolff NA, Abouhamed M, Verroust FJ et al (2006) Megalin-dependent internalization of cadmium-metallothionein and cytotoxicity in cultured renal proximal tubule cells. J Pharmacol Exp Ther 318:782–791
Wolff NA, Lee W-K, Abouhamed M et al (2008) Role of ARF6 in internalization of metal-binding proteins, metallothionein and transferrin, and cadmium-metallothionein toxicity in kidney proximal tubule cells. Toxicol Appl Pharmacol 230:78–85
Xie HQ, Kang YJ (2009) Role of cooper in angiogenesis and its medicinal implications. Curr Med Chem 16:1304–1314
Yamano T, Shimizu M, Noda T (1998) Comparative effects of repeated administration of cadmium on kidney, spleen, thymus, and bone marrow in 2-, 4-, and 8-month-old male Wistar rats. Toxicol Sci 46:393–402
Yang Y, Maret W, Vallee BL (2001) Differential fluorescence labeling of cysteinyl clusters uncovers high tissue levels of thionein. Proc Natl Acad Sci USA 98:5556–5559
Ye B, Maret W, Vallee BL (2001) Zinc metallothionein imported into liver mitochondria modulates respiration. Proc Natl Acad Sci USA 98:2317–2322
Yiin SJ, Chern CL, Sheu JY et al (1999) Cadmium-induced renal lipid peroxidation in rats and protection by selenium. J Toxicol Environ Health A 57:403–413
Yoshida M, Ohta H, Yamauchi Y et al (1998) Age-dependent changes in metallothionein levels in liver and kidney of the Japanese. Biol Trace Elem Res 63:167–175
Zalups RK, Ahmad S (2003) Molecular handling of cadmium in transporting epithelia. Toxicol Appl Pharmacol 186:163–188
Zangger K, Oz G, Armitage IM (2000) Re-evaluation of the binding of ATP to metallothionein. J Biol Chem 275:7534–7538
Zangger K, Shen G, Oz G, Otvos JD, Armitage IM (2001) Oxidative dimerization in metallothionein is a result of intermolecular disulphide bonds between cysteines in the alpha-domain. Biochem J 359:353–360

Zhang B, Georgiev O, Hagmann M et al (2003) Activity of metal-responsive transcription factor 1 by toxic heavy metals and H_2O_2 in vitro is modulated by metallothionein. Mol Cell Biol 23:8471–8485

Zheng H, Berman NEJ, Klaassen CD (1995) Chemical modulation of metallothinein I and III mRNA in mouse brain. Neurochem Int 27:43–58