# MINIREVIEW

# Metallothionein and Liver Cell Regeneration

M. GEORGE CHERIAN*,[1] AND Y. JAMES KANG†

*Department of Pathology, University of Western Ontario, London, Ontario N6A 5C1 Canada; and †Department of Medicine, University of Louisville School of Medicine, Louisville, Kentucky 40202

**Hepatocytes in adults are in a nonproliferative state but they have high capacity to regenerate within few hours after an injury. After partial hepatectomy or chemical injury, hepatocytes undergo a synchronized multistep process consisting of priming/initiation, proliferation, and termination. These distinct steps are essential for restoring the structure and functions of liver. The mechanisms involved in each of these steps of regeneration are well documented from various laboratories and are described in several reviews. We briefly describe these steps and the involvement of various cytokines and growth factors for cell regeneration in this short review. Liver cell regeneration may also involve stem cell proliferation. The regenerating cells require large amounts of zinc within a short time, and this requirement is met by induction of a zinc and copper binding protein, metallothionein (MT), during the priming step, soon after an injury. There are several reports on the transfer of zinc from MT to various metalloenzymes and transcription factors. Genetically modified mouse models have been used to study the involvement of interleukin (IL)-6 and tumor necrosis factor (TNF)-α in cell regeneration. The use of an MT-knockout mouse has enabled us to investigate the specific role of MT in liver regeneration after partial hepatectomy, chemical injury, and fibrosis. Several studies have suggested a defective liver regeneration after an injury in MT-knockout mice. There is cumulative evidence that indicates an essential role for MT in liver cell regeneration.** Exp Biol Med 231:138–144, 2006

**Key words:** cell injury; metallothionein; liver regeneration

## Introduction

There are several excellent reviews on liver regeneration after partial hepatectomy and tissue repair after chemically induced injury, and these reviews discuss in detail the various steps and mechanisms involved (1–4). In this minireview, we discuss briefly the various steps involved in liver regeneration, and the potential roles of metallothionein (MT) in cell regeneration and tissue repair in liver after chemical injury or after partial removal of liver in mouse models. Although the protective role of MT in cell injury is well studied, very little is known about the role of MT during cell regeneration. Recent studies in mice suggest that MT may play a role in repair and regeneration of injured liver (5–7). There are a few studies suggesting a similar role for MT in cell regeneration in central nervous system (8, 9) after injury.

## Liver Regeneration

In adult mammals, hepatocytes do not divide under normal conditions and have a long life span. However, it has been demonstrated as early as 1931, by Higgins and Anderson, that rat liver has a high capacity to regenerate within a few hours after partial hepatectomy (10). This rapid process of replacement of liver cells to restore the function is known as compensatory hyperplasia, and is an inherent property of liver repair after injury. Various mechanisms and distinct steps are involved in this compensatory cellular regeneration, and are studied in detailed in *in vivo* experiments using rat or mouse liver after partial hepatectomy or toxic injury (1–4). These studies have provided valuable information on different steps and mechanisms involved in the process of cell regeneration and tissue repair (Table 1). Soon after injury, hepatocytes undergo a synchronized multistep process consisting of priming/initiation, proliferation, and termination, and these processes are essential for restoring the structure and function of the liver. Because of the high capacity to regenerate, liver can grow back to approximately 100% within a few days after a partial

This research is supported by the National Institutes of Health grants CA 68125 and HL 59225 (to Y.J.K.) and by grants from Canadian Institute of Health Research (to M.G.C.).

[1] To whom correspondence should be addressed at 1151 Richmond Street, Health Science Addition, London, Ontario N6A 5C1, Canada. E-mail: mcherian@uwo.ca

1535-3702/06/2312-0138$15.00

**Table 1.** Steps Involved in Liver Cell Regeneration[a]

| |
|---|
| Liver cells ($G_0$) are in proliferative quiescence |
| Priming/initiation phase |
| Become replicative competence ($G_0$ to $G_1$/S phase) |
| Requires cytokines, such as IL-6 and TNF-α |
| IL-6 and TNF-α are made by nonparenchymal cells (sinusoidal endothelia, Kupffer cells, etc.) |
| The STAT family are transcription factors that are critical for cytokine signaling |
| Induction of several genes, including induction of MT synthesis and its nuclear localization |
| Proliferation phase: cell proliferation (expansion; synthesis of ECM protein) |
| In early stage of proliferation, JNK/c-Jun induces $G_0$ to $G_1$ transition with expression of cyclin D1 |
| HGF and TGF-α signaling control proliferation, which proceeds automatically under control of cyclins and cyclin-dependent kinases |
| The remodeling of growing lobule is modulated by Fas– and TNF-α–mediated apoptosis |
| A balance between hepatocyte proliferation and apoptosis occurs |
| Termination phase |
| The expansion phase is followed by a termination response by TGF-β and activins that inhibit DNA synthesis |
| TGF-β inhibits hepatocyte proliferation and induces apoptosis by a c-jun–independent mechanism |
| Activation of metalloproteinases can cause controlled degradation of certain extracellular matrix proteins and reshaping of the tissue |

[a] Adapted from Refs. 1 and 3.

hepatectomy or chemical injury. Hepatocytes, which are in a nonproliferative state, unlike other cells, can exit from the resting state of cell cycle ($G_0$) with external stimuli, enter $G_1$ phase, traverse to S phase, and undergo mitosis. This dynamic process in liver cells is controlled by expression of a large number of genes encoding various growth factors, cytokines, and transcription factors. The external signaling pathways may initiate the priming of liver cells within hours after a chemical injury or partial hepatectomy and are followed by initiation with growth factors and cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6. During the priming step, the quiescent liver cells from $G_0$ are traversed to $G_1$ phase and into a state of replicative competence. As described earlier in this article, induction of MT synthesis and its transient nuclear localization occur at this stage. Transcription factors, such as nuclear factor-κB (NF-κB) and signal transducer and activator of transcription protein 3 (STAT3), which are induced by TNF-α, play an important role in the initiation step in liver regeneration. In the next step, the stimulation *via* mitogens, such as hepatocyte growth factor (HGF), transforming growth factor (TGF)-α and epidermal growth factor (EGF), occurs by the mitogens binding to receptors on hepatocytes, which initiates proliferation, and allows the liver cells to enter into the S phase of the cell cycle. The final stage of termination of the cell cycle is achieved by upregulation of TGF-β and activin, which can suppress epithelial cell growth. During this final stage of tissue repair, metalloproteinases are activated to complete the tissue repair process by degradation of extracellular matrix, and reshaping of the tissue. Thus, during the process of liver cell regeneration, the cells are at a high metabolic state, with an increased requirement for zinc and copper. Although compensatory tissue repair can occur in other organs after chemical injury or in certain disease states, it is more predominant in the liver. The basic steps involved in cell regeneration and tissue repair in most organs may be similar to those described in Table 1, but the initiation factors and the time period for regeneration may differ from cell type to cell type, and from organ to organ. Animal studies have suggested that various factors, such as species, strain, age, nutrition, types of cell injury, and disease states, can modulate both the rate and degree of cell regeneration in liver (11, 12). For example, in diabetic rats, the regeneration of liver after chemical injury is slower than in controls. Because liver has very high capacity to regenerate, most of studies on compensatory cell regeneration are undertaken in liver (1–4, 10).

## MT and its Potential Biological Functions

Mammalian cells demonstrate complex but distinct responses during repair and cell regeneration after an injury. One of these responses is induction of MT. MT was first isolated as a cadmium-binding protein from horse kidney approximately five decades ago, and was later characterized as a low molecular weight protein with a high cysteine content and a high affinity for divalent essential metals, such as zinc and copper, and nonessential metals, such as cadmium and mercury (13, 14). The three-dimensional structure of MT as elucidated by x-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy has demonstrated that the metals are bound tetrahedrally to cysteines in two distinct clusters with high thermodynamic but low kinetic stability. Thus, the chemical structure of MT suggests that although the metals are bound very tightly, the transfer of metals from MT to other proteins is possible under various conditions. A number of potential biological functions of MT were proposed soon after its characterization. These functions include protection against certain metal toxicity, DNA damage, radiation and oxidative injury, and a zinc-donating role for several metalloproteins, transcription factors, and enzymes. Thus, the ubiquitous

expression of MT may have multiple functions. Organ-specific overexpression of MT in transgenic mice has demonstrated its protective effects in kidney, liver, heart, pancreas, and also against oxidative stress (15–18). Studies using MT-null (MT knockout) mice have confirmed the protective effects of MT against metal toxicity and oxidative stress (15, 18). The essentiality of MT for life remains debatable, however, MT is likely required for cell repair and regeneration after certain types of stress conditions, such as partial hepatectomy or chemical injury. It is also known that the MT transcription factor (MTF)-1, that is responsible for MT induction in mammalian cells is essential because the deletion of the *Mtf*-1 gene is lethal to the mouse embryo (19). This short review will focus on some of the recent studies that support a role for MT in the repair of liver injury and the regeneration of cells.

## Role of MT in Zinc Transport During Stress Conditions

Cells in a living organism are constantly exposed to various stress conditions, such as oxidative stress and toxic agents. However, most cells have endogenous protective mechanisms, including the ability to synthesize various antioxidants and/or enzymes to protect them from different toxic insults. Cellular recovery and regeneration after an injury are important processes for the survival of the organism and may depend on various factors, such as the type of injury, the type of cells, and their nutritional status (11, 12). For cells to protect themselves against oxidative stress and to regenerate after injury, there is a high requirement for zinc and copper to activate metalloenzymes, such as superoxide dismutase. Because most of the metals (except alkaline metals) are intracellularly bound to proteins or amino acids in the cell, zinc and copper have to be released from metalloproteins, such as MT. The induced synthesis of MT in cells within hours after an injury is a typical example of a cellular defense mechanism, and suggests that MT can act as a chaperone to provide zinc and copper to various transcription factors, metalloenzymes, and growth factors that are required for repair and rapid cell growth (20–22). Because of the high content of cysteine residues, MT may also act as an antioxidant under certain conditions to protect the cells from oxidative stress (23, 24). The mechanism of release of metals (zinc and copper) bound to cysteines in the two clusters of MT is not yet well understood. However, it has been suggested that disulfides, such as oxidized glutathione, and oxidative stress can provide an oxidoreductive environment in the cell for oxidation of MT and release of zinc to apo-metalloproteins (25). In addition, the transient nuclear localization of MT in cells at the $G_1$-to-S phase transition in rapidly proliferating cells, and also after cell injury, indicates that the high demand of zinc for DNA repair and synthesis can be met by induction of MT synthesis and sequestration of zinc, and transfer of zinc to enzymes (26). The nuclear presence of MT in cells can be prolonged by cell cycle arrest at S phase with aphidicolin, and suggests a role for MT in cell proliferation (27). Although *in vitro* studies have demonstrated transfer of zinc from MT to apo-metalloenzymes and zinc-finger transcription factors, such as Sp1, TFIIA, estrogen receptors, and tramtak, there is little information on the transfer of zinc from MT *in vivo* (28, 29). A recent study has demonstrated that MT can transfer zinc to mitochondrial aconitase through a direct interaction in mouse heart (30). However, other adaptive mechanisms may be involved in activation of these enzymes in MT-knockout mice and cells without presence of MT. In addition, other studies using MT-I and MT-II knockout mice have shown more severe cell damage than wild-type mice after chemical exposure in the absence of MT-I and MT-II and lacking a primary protective mechanism. These studies also suggested a zinc storage and donor role for MT in liver cell regeneration after chemical injury, alcoholic liver fibrosis, and after partial hepatectomy (5–7).

## MT Expression in Cell Injury and Role in Regeneration

Because liver has high levels of zinc- and copper-bound MT and has a high capacity to regenerate, it is commonly used as a model to study the role of MT in tissue regeneration. Two different experimental conditions are commonly used to study cell regeneration in the liver: (i) partial hepatectomy (1–4, 10); and (ii) chemical injury (12). The induction of MT synthesis and its transient nuclear localization in rodent liver within hours after partial hepatectomy support such a role in acute stress conditions (31). Previous studies have shown that induction of MT synthesis can protect animals from hepatotoxicity of several chemicals, such as ethanol, carbon tetrachloride, acetaminophen, and cadmium (18). The generation of an MT-knockout mouse line with inactivation of the two major MT genes (MT-I and MT-II) has provided an excellent animal model to study the specific role of MT in metal toxicity and other cellular functions (32). The high levels of MT expression and its presence in the nucleus during rapid cell growth and development as well as in highly proliferative tumors indicate a function for MT in cell growth (33). However, the steps in which MT may play a role during liver cell regeneration are still unclear. It is important to critically evaluate the experimental evidence for the biological roles of MT in protecting cells from injury and also during recovery stages of cell regeneration.

As described in Table 1, during the priming/initiating stage of liver cell regeneration, both cytokines IL-6 and TNF-$\alpha$ are involved in converting the quiescence liver cells to replicative competence. After cell injury, the cytokine levels are increased and the cytokines bind to receptors on injured hepatocytes, and synthesis of MT is increased concomitantly with expression of a number of other genes. Studies using cytokine-knockout mice have provided useful

information regarding the specific roles of IL-6 and TNF-α on MT synthesis after partial hepatectomy (34, 35). These are summarized in Table 2. After partial hepatectomy, defective hepatocyte regeneration and liver failure were observed in IL-6–deficient mice (34). Moreover, $G_1$ phase abnormalities, including absence of STAT3 activation and depression of AP-1 and cyclin D1 expression were observed in these mice. Another study showed that the hepatic MT levels were decreased significantly in IL-6–knockout mice as compared with wild-type mice after partial hepatectomy; but the IL-6–knockout mice still showed some residual capacity to synthesize hepatic MT (35). Pretreatment of IL-6–knockout mice with IL-6 restored both hepatic MT synthesis and liver regeneration to control levels, demonstrating that IL-6 is one of the major inducers of hepatic MT synthesis and a critical factor for liver regeneration after partial hepatectomy. In this study, the serum levels of both IL-6 and TNF-α were increased within hours after partial hepatectomy in wild-type mice, whereas the TNF-α serum level in IL-6–knockout mice was approximately two times higher than that in wild-type mice. Although it is well known that TNF-α is one of the cytokines produced after metal exposure and is known to induce MT synthesis, its role in cell regeneration is unclear. However, it has been suggested that, during liver regeneration, the TNF-α–mediated NF-κB activation may be one of the key events (2). Analysis of liver regeneration in mice lacking TNF-α receptors (TNFR) Type-1 or Type-2 showed that TNFR-1 is required for the initiation of liver regeneration after partial hepatectomy, whereas absence of TNFR-2 had little effect (36). Mice lacking TNFR-1 had defective DNA synthesis and a massive lipid accumulation in hepatocytes, whereas TNFR-2–knockout mice had a normal liver structure and similar DNA replication to wild-type mice. Serum levels of IL-6 in TNFR-1–knockout mice were lower than wild-type and TNFR-2–knockout mice after partial hepatectomy (Table 2). One of the effects of TNF-α is induction of IL-6. Moreover, administration of IL-6 to TNFR-1–deficient mice restored some of the observed deficiencies in liver regeneration (36). A preliminary study in TNF-α–knockout mice (37) showed no difference in hepatic MT levels in these mice as compared with wild-type mice after partial hepatectomy, suggesting that TNF-α may play a lesser role than IL-6 in controlling MT synthesis in mice during liver regeneration. However, a recent *in vitro* study using murine hepatoma and macrophage cell lines showed that antibodies to both TNF-α and IL-6 could inhibit MT synthesis, suggesting that both cytokines may be involved in induction of MT synthesis in certain cells (38). All of these studies suggest that induction of MT synthesis is an early event in liver regeneration after injury or resection, and recent results using MT-knockout mice suggest that MT synthesis is essential for normal cell proliferation after partial hepatectomy and chemical liver injury (Table 2; Refs. 5–7).

In two recent studies, the potential role of MT in liver regeneration was investigated in wild-type and in MT-I– and MT-II–knockout mice (the two major MT genes were inactivated) using two different models: (i) surgical resection of 35% of liver by partial hepatectomy and (ii) acute liver damage after intraperitoneal injection with thioacetamide, a liver toxin (5, 7). In wild-type mice, both treatments resulted in increased induction of MT synthesis in liver within 12 hrs, with a transient nuclear translocalization. Hepatocyte proliferation rate, as determined by both argyrophilic nucleolar organizing region (AgNOR) with silver staining and proliferating cell nuclear antigen (PCNA) immunohistochemistry, was high (40%–55% higher than sham-operated controls) between 48 and 72 hrs after the partial hepatectomy or after treatment with 125 mg/kg thioacetamide in wild-type mice. In MT-knockout mice, there was no change in the low background detectable hepatic levels of MT and no detectable immunoreactivity for MT. Hepatic proliferation index levels for MT-knockout mice were low (approximately 18%–25%) at every time point examined after the partial hepatectomy, and did not change much during the liver regeneration period. Similar results were observed in cell proliferation in chemical injury after treatment with thioacetamide. These two studies demonstrated that, in the absence of MT, the cell proliferation is very low in MT-knockout mice after liver resection or chemical injury, thereby, suggesting an essential role for MT in cell proliferation and liver regeneration.

There are however, a few differences between these two experimental models, and the cell proliferation rate may vary depending on various factors, such as cellular content of antioxidants and the type of cell injury. In the partial hepatectomy model, there was no histologic evidence for apoptosis or necrosis in the remaining liver of wild-type and MT-knockout mice, whereas, after thioacetamide treatment, there was severe hemorrhagic necrosis in the liver, with more severe damage in the livers of MT-knockout mice. Most of the liver damage after thioacetamide treatment was caused by free-radical generation and oxidative stress, and analysis of glutathione showed an early time-dependant depletion of reduced glutathione levels in MT-knockout mice as compared with wild-type mice. The hepatic levels of oxidized glutathione and lipid peroxidation were much higher in MT-knockout mice as compared with wild-type mice. The recovery of the reduced glutathione levels in MT-knockout mice was also slow after chemical injury. The results suggest that, after a chemical injury, the initial step is the repair process, which is followed by initiation of the cell regeneration. Several cellular factors and the severity of injury can affect the ability of cells to initiate cell repair and regeneration. In the partial hepatectomy model, there is little cell injury, and the regeneration process is initiated immediately after liver resection. Therefore, a slower recovery of the injured cells may be expected in MT-knockout mice than in wild-type mice after a chemical injury. Irrespective of these differences, the very low proliferation rate observed in MT-knockout mice in both

**Table 2.** Cellular Changes After Partial Hepatectomy[a]

| Type of mice | MT-expression | Cell regeneration | IL-6 serum | TNF-α serum |
|---|---|---|---|---|
| Control | High | Normal | Increased | Increased |
| MT KO | None | Decreased | No data | No data |
| IL-6 KO | Low | Defective | None | Increased |
| TNF-α KO | Normal | No data | No data | None |
| TNFR-1 KO | No data | Defective | Decreased | Increased |
| TNFR-2 KO | No data | Normal | Normal | Normal |

[a] Summary of data adopted from Refs. 34, 35, 36, and 37.

models suggests a positive role for MT in cell regeneration in addition to its antioxidant function. However, the exact role of MT in different steps in regeneration of liver is not yet understood. From these results, we can only speculate that the role of MT may be related to the supply of zinc for zinc-finger transcription factors and enzymes required for DNA repair and synthesis during cell regeneration.

The role of MT in liver regeneration is further supported by a MT gene-therapy study (6). In this study, a 4-week treatment of wild-type mice with carbon tetrachloride caused a reversible liver fibrosis with high levels of MT, whereas, in MT-knockout mice, such treatment resulted in irreversible fibrosis. When wild-type mice were treated for 8 weeks with carbon tetrachloride, they also developed an irreversible fibrosis with low hepatic levels of MT. The most convincing evidence for a specific role for MT in liver regeneration after a chemical injury comes from gene-therapy experiments in these mice. When the mice with irreversible liver fibrosis were treated with adenoviral delivery of the human *MT*-II gene through intravenous injection, the fibrosis was reversed, with increased hepatocyte regeneration within 3 days. The hepatic MT elevation in these mice was accompanied by increased activities of collagenases in the liver. The most probable mechanism involved is the activation of collagenases by zinc released from MT. The specific role of MT in cell regeneration and tissue repair is likely related to its role as a potential metal donor for several transcription factors and metalloenzymes.

**Table 3.** Enzymes and Transcription Factors Activated by MT

| Enzymes | References |
|---|---|
| Aconitase (mitochondrial) | 20 |
| Aldolase (yeast) | 10 |
| Alkaline phosphatase (*Escherichia coli*) | 10 |
| Bovine carboxypeptidase | 42 |
| Carbonic anhydrase (erythrocyte) | 10 |
| Collagenase (metalloproteinase 13) | 22 |
| Sorbitol dehydrogenase | 43 |
| Thermolysin | 10 |
| **Transcription factors** | |
| Estrogen receptors | 12 |
| Gal4 | 44 |
| Sp1 | 45, 46 |
| TFIIIA | 11, 47 |
| Tramtrack | 48 |

Because MT can bind metals with high affinity, its high expression will serve as a reservoir for essential metals, such as zinc and copper, during mammalian development and cell proliferation. The high levels of zinc and copper bound to MT in livers during gestation and the early neonatal period as well as in proliferative tumors support this speculation. Studies using blue crab, *Callinectes sapidus,* have shown that there are changes in copper bound to hemocyanin, the oxygen-carrying protein in crustaceans and MT in hepatopancreas during different stages of the molt cycle (39). The induced synthesis of MT and the sequestration of copper by MT can be observed during the early stage of the molt cycle. The copper stored in MT during the molt stage was transferred back to hemocyanin after molting was completed. Thus, the induction of MT during certain stages in their development serves as a copper reservoir. This is one of the best demonstrations of the transfer of copper from MT to a functional protein, such as hemocyanin, during a physiologic process. However, the release of metals from MT or their transfer to other functional proteins and enzymes need further investigation.

Under oxidative and nitrosative stress conditions, the metal-binding clusters in MT can be disrupted, resulting in the release of metals. It has been shown that zinc can be released from MT after treatment with S-nitrosocysteine (40) or nitric oxide (41), and increase the intracellular labile zinc levels, which can be easily transferred to other proteins and enzymes. NMR spectroscopic studies demonstrate specific interaction of nitric oxide with MT at the three-metal cluster in the amino-terminal domain of MT, leading to the release of zinc from the amino-terminal domain of MT (41). In addition, the formation of disulfides of glutathione and other thiols within the cells can provide an oxidoreductive environment that may favor oxidation of MT to release metals whenever required (25). Thus, the redox conversions of cysteines in MT are critical for binding and release of metals. It is also known that the metals bound to the clusters in MT are not static but are constantly exchanging metals within the cluster and from cluster to cluster, suggesting a dynamic process of MT binding to

metals. Therefore, induction of thionein or metal requiring apo-proteins or apo-enzymes can exchange zinc and copper from MT and other metal binding proteins. To donate zinc or copper from MT, a direct interaction may be needed, as demonstrated in mitochondrial aconitase (30), or it could be a concentration-dependant process. Whatever may be the mechanism of the metal transfer from MT, because of the high capacity and affinity for zinc and copper, MT should be considered as an excellent source of these metals stored in a nontoxic form within the cell. The defective hepatocyte proliferation in MT-knockout mice after partial hepatectomy and chemical injury demonstrate that the high requirement for zinc and copper during cell repair and regeneration cannot be met in the absence of MT.

The recent studies indicate that hepatic MT expression can activate various metalloenzymes and transcription factors required for cell regeneration and tissue repair, suggesting that MT may act as an essential growth factor during regeneration of injured cells. These enzymes and transcription factors are listed in Table 3.

## Summary

During the last five decades, extensive data have been accumulated on the structure and expression of various isoforms of MT in different organ systems and cell types. These studies demonstrate the unique structure of MT, with its high affinity for certain divalent metals, and also its functions as a metal-storage protein and antioxidant. In addition, the ability of MT to protect against metal toxicity, free radicals, radiation, and DNA damage may be closely related to its structure. The generation of transgenic mice overexpressing MT in various organs and MT-knockout mice provided useful models to investigate the specific biological role of MT in various cell functions. Recent studies using these models suggest that MT may play a general role in energy metabolism and a specific role in the transfer of essential metals, such as zinc and copper, to various metalloenzymes and transcription factors, which are required during tissue repair, cell proliferation, and regeneration. In the process of repairing liver injury and hepatic regeneration, MT is required. Mice with inactive MT-I and MT-II genes are sensitive to the toxicity of chemicals, and their livers cannot regenerate after chemical injury and partial hepatectomy.


1. Zimmermann A. Regulation of liver regeneration. Nephrol Dial Transplant 19:6–10, 2004.
2. Michalopoulos GK, DeFrances M. Liver regeneration. Adv Biochem Engin Biotechnol 95:101–134, 2005.
3. Fausto N. Liver regeneration. J Hepatol 32:19–31, 2000.
4. Koniaris LG, McKillop IH, Schwartz SI, Zimmers TA. Liver regeneration. J Am Coll Surg 197:634–652, 2003.
5. Oliver JR, Jiang S, Cherian MG. Augmented hepatic injury followed by impaired regeneration in metallothionein-I/II knockout mice after treatment with thioacetamide. Toxicol Appl Pharmacol (in press), 2005.
6. Jiang Y, Kang YJ. Metallothionein gene therapy for chemical-induced liver fibrosis in mice. Mole Therapy 10:1130–1139, 2004.
7. Oliver JR, Mara TW, Cherian MG. Impaired hepatic regeneration in metallothionein-I/II knockout mice after partial hepatectomy. Exp Biol Med 230:61–67, 2005.
8. West AK, Chuah MI, Vickers JC, Chung RS. Protective role of metallothionein in the injured mammalian brain. Rev Neurosci 15:157–166, 2004.
9. Chung RS, West AK. A role for extracellular metallothionein in CNS injury and repair. Neuroscience 123:595–599, 2004.
10. Higgins GM, Anderson RM. Experimental Pathology of liver. 1. Restoration of the liver of white rat following partial surgical removal. Arch Pathol 12:186–202, 1931.
11. Mehendale HM. Toxicodynamics of low level toxicant interactions of biological significance: inhibition of tissue repair. Toxicology 105:251–266, 1995.
12. Mehendale HM. Tissue repair: an important determinant of final outcome of toxicant-induced injury. Toxicol Pathol 33:41–51, 2005.
13. Margoshes M, Valle BL. A cadmium protein from equine kidney cortex. J Am Chem Soc 79:4813–4814, 1957.
14. Kagi JHR, Valle BL. Metallothionein: a cadmium and zinc containing protein from equine renal cortex. J Biol Chem 235:3460–3465, 1960.
15. Liu Y, Liu J, Iszard MB, Andrews GK, Palmiter RD, Klaassen CD. Transgenic mice that overexpress metallothionein-I are protected from cadmium lethality and hepatotoxicity. Toxicol Appl Pharmacol 135: 222–228, 1995.
16. Kang YJ, Chen Y, Yu A, McVoss-Cowan M, Epstein PN. Over-expression of metallothionein in the heart of transgenic mice suppresses doxorubicin cardiotoxicity. J Clin Invest 100:1501–1506, 1997.
17. Chen H, Carlson EC, Pellet L, Moritz JT, Epstein PN. Overexpression of metallothionein in pancreatic beta cells reduces streptozotocin-induced DNA damage and diabetes. Diabetes 50:2040–2046, 2001.
18. Klaassen CD, Liu J, Choudhuri S. Metallothionein: an intracellular protein to protect against cadmium toxicity. Ann Rev Pharmacol Toxicol 39:267–294, 1999.
19. Guncs C, Heuchel R, Georgiev O, Muller KH, Lichtlen P, Bluthmann H, Marino S, Aguzzi A, Schaffner W. Embryonic lethality and liver degeneration in mice lacking the metal-responsive transcriptional activator MTF-1. EMBO J 17:2846–2854, 1998.
20. Udom AO, Brady FO. Reactivation in vitro of zinc-requiring apo-enzymes by rat liver zinc-thionein. Biochem J 187:329–335, 1980.
21. Zeng J, Vallee BL, Kagi JH. Zinc transfer from transcription factor IIIA fingers to thionein clusters. Proc Natl Acad Sci U S A 88:9984–9988, 1991.
22. Cano-Gauci DF, Sarkar B. Reversible zinc exchange between metallothionein and the estrogen receptor zinc finger. FEBS Lett 386:1–4, 1996.
23. Thornally PJ, Vasak M. Possible role of metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. Biochim Biophys Acta 827:36–44, 1985.
24. Sato M, Bremner I. Oxygen free radicals and metallothionein. Free Radic Biol Med 14:325–337, 1993.
25. Maret W. Oxidative metal release from metallothionein via zinc-thiol/disulfide interchange. Proc Natl Acad Sci U S A 91:237–241, 1994.
26. Cherian MG, Apostolova MD. Nuclear localization of metallothionein during cell proliferation and differentiation. Cell Mol Biol 46:347–356, 2000.
27. Apostolova MD, Cherian MG. Delay of M-phase onset by aphidicolin can retain the nuclear localization of zinc and metallothionein in 3T3-L1 fibroblasts. J Cell Physiol 183:247–253, 2000.
28. Posewitz MC, Wilcox DE. Properties of the Sp1 zinc finger 3 peptide:

coordination chemistry, redox reactions, and metal binding competition with metallothionein. Chem Res Toxicol 8:1020–1028, 1995.
29. Romero-Isart N, Vasak M. Advances in the structure and chemistry of metallothioneins. J Inorg Biochem 88:388–396, 2002.
30. Feng W, Cai J, Pierce WM, Franklin RB, Maret W, Benz FW, Kang YJ. Metallothionein transfers zinc to mitochondrial aconitase through a direct interaction in mouse hearts. Biochim Biophys Res Comm 332: 853–858, 2005.
31. Tohyama C, Suzuki JS, Hemelraad J, Nishimura N. Nishimura H. Induction of metallothionein and its localization in the nucleus of rat hepatocytes after partial hepatectomy. Hepatology 18:1193–1201, 1993.
32. Masters BA, Kelly EJ, Quaife CJ, Brinster RL, Palmiter RD. Targeted disruption of metallothionein I and II genes increases sensitivity to cadmium. Proc Natl Acad Sci U S A 91:584–588, 1994.
33. Cherian MG, Jayasuriya A, Bay BH. Metallothioneins in human tumors and potential roles in carcinogenesis. Mutat Res 533:201–209, 2003.
34. Cressman DE,Greenbaum LE, DeAngelis RA, Cilberto G, Furth EE, Poli V, Taub R. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 274:1379–1383, 1996.
35. Molotkov A, Nishimura N, Satoh M, Tohyama C. Role of IL-6 in the induction of hepatic metallothionein in mice after partial hepatectomy. Life Sciences 66:963–970, 2000.
36. Yamada Y, Webber EM, Kirillova I, Peschon JJ, Fausto N. Analysis of liver regeneration in mice lacking type 1 or type 2 tumor necrosis factor receptor: requirement for type 1 but not type 2 receptor. Hepatology 28: 959–970, 1998.
37. Oliver JR. Role of metallothionein in hepatic injury and regeneration. MSc thesis, the University of Western Ontario, London, Ontario, Canada. 2005.
38. Jeong HG, Kim HG, Hwang YP. Involvement of cytokines in the hepatic expression of metallothionein by ursolic acid. Toxicol Lett 155: 369–376, 2005.
39. Brouwer M, Syring R, Brouwer TH. Role of a copper-specific metallothionein of the blue crab, *Callinectes sapidus*, in copper metabolism associated with degradation and synthesis of hemocyanin. J Inorg Biochem 88:228–239, 2002.
40. St Croix CM, Wasserloos KJ, Dineley KE, Reynolds IJ, Levitan ES, Pitt BR. Nitric oxide-induced changes in intracellular zinc homeostasis are mediated by metallothionein/thionein. Am J Physiol 282:185–192, 2002.
41. Zangger K, Oz G, Haslinger E, Kunert O, Armitage IM. Nitric oxide selectively releases metals from the amino-terminal domain of metallothioneins: potential role at inflammatory sites. FASEB J 15:1303–1305, 2001.
42. Jacob C, Maret W, Vallee BL. Control of zinc transfer between thionein, metallothionein, and zinc proteins. Proc Natl Acad Sci U S A 95:3489–3494, 1998.
43. Jiang LJ, Maret W, Vallee BL. The glutathione redox couple modulates zinc transfer from metallothionein to zinc depleted sorbitol dehydrogenase. Proc Natl Acad Sci U S A 95:3483–3488, 1998.
44. Maret W, Larsen KS, Vallee BL. Co-ordination dynamics of biological zinc "clusters" in metallothioneins and in the DNA binding domain of the transcription factor Gal4. Proc Natl Acad Sci U S A 94:2233–2237, 1997.
45. Posewitz MC, Wilcox DE. Properties of the Sp1 zinc finger 3 peptide: coordination chemistry, redox reactions and metal binding competition with metallothionein. Chem Res Toxicol 8:1020–1028, 1995.
46. Zeng J, Heuchel R, Schaffner W, Kagi JH. Thionein (apometallothionein) can modulate DNA binding and transcription activation by zinc finger containing factor Sp1. FEBS Lett 279:310–312, 1991.
47. Huang M, Shaw III CF, Petering DH. Interprotein metal exchange between transcription factor IIIA and metallothionein. J Inorg Biochem 98:639–648, 2004.
48. Roesijadi G, Bogumil R, Vasak M,Kagi JH. Modulation of DNA binding of a tramtrack zinc finger peptide by metallothionein-thionein conjugate pair. J Biol Chem 273:17425–17432, 1998.