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Metallothionein Genes Research Literatures

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Abstract: Metallothionein (MT) is a family of cysteine-rich, low molecular weight (MW ranging from 500 to 14000 Da) proteins. They are localized to the membrane of the Golgi apparatus. MTs have the capacity to bind both physiological (such as zinc, copper, selenium) and xenobiotic (such as cadmium, mercury, silver, arsenic) heavy metals through the thiol group of its cysteine residues, which represent nearly 30% of its constituent amino acid residues. MT was discovered in 1957 by Vallee and Margoshe from purification of a Cd-binding protein from horse (equine) renal cortex. MT plays a role in the protection against metal toxicity and oxidative stress, and is involved in zinc and copper regulation. There are four main isoforms expressed in humans (family 1, see chart below): MT1 (subtypes A, B, E, F, G, H, L, M, X), MT2, MT3, and MT4. In the human body, large quantities are synthesised primarily in the liver and kidneys. Their production is dependent on availability of the dietary minerals such as zinc, copper, and selenium, as well as the amino acids histidine and cysteine. Metallothioneins are rich in thiols, causing them to bind a number of trace metals. Metallothionein binds several Zn ions. One of few eukaryotic proteins distinguished as having a role in substantial metal detoxification. Zinc and Cadmium are tetrahedrally coordinated to cysteine residues, each metallothionein protein molecule may bind up to 7 atoms of Zn or Cd. The biosynthesis of metallothionein appeared to have increased by several-fold throughout oxidative stress to shield the cells against cytotoxicity and DNA damage. Metallothionein biosynthesis can also be induced by certain agents or conditions, for example, hormones, pharmaceuticals, alcohols, other substance treatments and many more. Metallothionein is a cytoplasmic protein, in an adult liver, it is localized mainly in the cytoplasm. In human fetus, metallothionein is localized in hepatocyte nuclei. This article introduces recent research reports as references in the related studies.
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Key words: Metallothionein; Genes; cell; life; research; literature

Introduction

Metallothionein (MT) is a family of cysteine-rich, low molecular weight (MW ranging from 500 to 14000 Da) proteins. They are localized to the membrane of the Golgi apparatus. MTs have the capacity to bind both physiological (such as zinc, copper, selenium) and xenobiotic (such as cadmium, mercury, silver, arsenic) heavy metals through the thiol group of its cysteine residues, which represent nearly 30% of its constituent amino acid residues. MT was discovered in 1957 by Vallee and Margoshe from purification of a Cd-binding protein from horse (equine) renal cortex. MT plays a role in the protection against metal toxicity and oxidative stress, and is involved in zinc and copper regulation. There are four main isoforms expressed in humans (family 1, see chart below): MT1 (subtypes A, B, E, F, G, H, L, M, X), MT2, MT3, and MT4. In the human body, large quantities are synthesised primarily in the liver and kidneys. Their production is dependent on availability of the dietary minerals such as zinc, copper, and selenium, as well as the amino acids histidine and cysteine. Metallothioneins are rich in thiols, causing them to bind a number of trace metals. Metallothionein binds several Zn ions. One of few eukaryotic proteins distinguished as having a role in substantial metal detoxification. Zinc and Cadmium are tetrahedrally coordinated to cysteine residues, each metallothionein protein molecule may bind up to 7 atoms of Zn or Cd. The biosynthesis of metallothionein appeared to have increased by several-fold throughout oxidative stress to shield the cells against cytotoxicity and DNA damage. Metallothionein biosynthesis can also be induced by certain agents or conditions, for example, hormones, pharmaceuticals, alcohols, other substance treatments and many more. Metallothionein is a cytoplasmic protein, in an adult liver, it is localized mainly in the cytoplasm. In human fetus, metallothionein is localized in hepatocyte nuclei (https://en.wikipedia.org/wiki/Metallothionein). This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Abdel-Mageed, A. B., et al. (2003). "Erythropoietin-induced metallothionein gene expression: role in proliferation of K562 cells." Exp Biol Med (Maywood) 228(9): 1033-1039.

Recent evidence has demonstrated an appreciable expression of metallothionein (MT) in erythrocytes. However, the induction of the MT protein by hematopoietic growth factors and its subsequent functional significance on clonal expansion or differentiation of erythroid progenitor cells remain elusive. We therefore examined the effects of growth factors erythropoietin (EPO), granulocyte-monocyte colony-stimulating factor (GM-CSF), and interleukin-3 (IL-3) on MT gene expression in erythroid progenitor K562 cell line. EPO, but not IL-3 or GM-CSF, induced a 3-fold increase in MT transcripts in K562 cells. MT induction was associated with EPO-induced cellular proliferation, suggesting a specific role for MT induction by EPO in erythroid progenitor cells. However, EPO- or sodium butyrate-induced differentiation as monitored by hemoglobin formation was inhibited in K562 cells stably transfected with an expression vector containing human MT-IIA gene. This inhibition of differentiation was partially reversed in these cells by an MT antisense phosphorothioate oligonucleotide. Furthermore, the MT-induced inhibition of differentiation was associated with downregulation of EPO receptor transcripts in K562 cells. These data suggest that, among growth factors required for erythropoiesis, EPO is a potent inducer of MT, and that MT may plays a significant role in EPO-induced proliferation, but not in the erythroid-specific differentiation of the progenitor cells.

Abshire, M. K., et al. (1996). "Induction of c-myc and c-jun proto-oncogene expression in rat L6 myoblasts by cadmium is inhibited by zinc preinduction of the metallothionein gene." J Toxicol Environ Health 48(4): 359-377.

Certain proto-oncogenes transfer growth regulatory signals from the cell surface to the nucleus. These genes often show activation soon after cells are exposed to mitogenic stimulation but can also be activated as a nonmitogenic stress response. Cadmium (Cd) is a carcinogenic metal in humans and rodents and, though its mechanism of action is unknown, it could involve activation of such proto-oncogenes. Metallothionein (MT), a metal-inducible protein that binds Cd, can protect against many aspects of Cd toxicity, including genotoxicity and possibly carcinogenesis. Thus, the effects of Cd on expression of c-myc and c-jun in rat L6 myoblasts, and the effect of preactivation of the MT gene by Zn treatment on such oncogene expression, were studied. MT protein levels were determined by the Cd-heme assay, and MT, c-myc, and c-jun mRNA levels were measured using oligonucleotide hybridization and standardized to beta-actin levels. Cd (5 microM CdCl2, 0-30 h) stimulated both c-myc and c-jun mRNA expression. An initial peak of activation of c-myc expression occurred 2 h after initiation of Cd exposure, and levels remained elevated throughout the assessment period. Zn pretreatment markedly reduced the activation of c-myc expression by Cd compared to cells not receiving Zn pretreatment. Cd treatment increased c-jun mRNA levels by up to 3.5-fold. Again, Zn pretreatment markedly reduced Cd-induced activation of c-jun expression as minimal increases occurred with Cd exposures of < or = 1 h, but otherwise the Zn pretreatment prevented activation of c-jun. The Zn pretreatment elevated MT protein levels > 5-fold over control at the point of Cd exposure, but Cd exposure did not further elevate these Zn-induced MT levels. Similarly, Zn pretreatment did not result in increased relative MT mRNA levels above Cd exposure alone at various time points after Cd exposure. Therefore, Zn pretreatment, possibly by providing elevated MT protein levels at the point of Cd exposure, inhibited the Cd-induced c-myc and c-jun proto-oncogene expression. The extent of Cd-induced proto-oncogene activation thus may be limited by the presence of cellular MT.

Ahmadi, N., et al. (2003). "The promoter of a metallothionein-like gene from the tropical tree Casuarina glauca is active in both annual dicotyledonous and monocotyledonous plants." Transgenic Res 12(3): 271-281.

A chimeric gene consisting of the beta-glucuronidase (gusA) reporter gene under the control of the metallothionein-like promoter cgMT1 from the tropical tree Casuarina glauca was introduced into Nicotiana tabacum via Agrobacterium tumefaciens and into Oryza sativa by particle bombardment. The strongest histochemical staining for GUS activity was observed in the root system of the transgenic plants, and especially in lateral roots. In contrast, a relatively low level of reporter gene expression was seen in the aerial tissues and GUS staining was located mainly in the plant vascular system. The average ratio of GUS activity between root and leaf was found to be 13:1 in tobacco and 1.5:1 in rice. The pattern of cgMT1 promoter activity in floral organs was found to be different in tobacco and rice. High levels of gusA gene expression were detected in the ovules, pollen grains and tapetum, whereas in rice PcgMT1 directs expression to the vascular system of the floral organs. These results suggest that PcgMT1 is potentially useful in molecular breeding to express genes of interest whose products are preferentially needed in roots.

Alam, J. and A. Smith (1992). "Heme-hemopexin-mediated induction of metallothionein gene expression." J Biol Chem 267(23): 16379-16384.

Hemopexin-mediated heme transport into

mouse hepatoma (Hepa) cells and human promyelocytic (HL-60) cells stimulates the expression of heme oxygenase via transcriptional activation (Alam, J., and Smith, A. (1989) J. Biol. Chem. 264, 17637-17640). Incubation of both these cell types in serum-free medium containing heme-hemopexin is shown here also to increase the steady-state level of metallothionein (MT) mRNA in a time- and dose-dependent manner. Heme-hemopexin is a far more effective inducer (12-fold) of the MT isozyme 1 (MT-1) in Hepa cells than nonprotein-bound heme (4-fold). Apohemopexin has no effect on MT-1 expression, and incubation with heme-hemopexin of mouse L fibroblasts that lack hemopexin receptors does not affect MT-1 expression. Thus, an interaction between the heme-hemopexin complex and its receptor is necessary for increased accumulation of MT-1 transcripts. In vitro nuclear "run-on" analysis indicates that the heme-hemopexin-mediated accumulation of MT-1 mRNA is regulated primarily at the level of initiation of transcription. A highly labile protein is required for constitutive MT-1 gene expression and acts to repress transcription. Transcriptional activation by heme or metals may require decreased concentrations or inactivation of the repressor as well as an additional inducer-specific trans-acting factor. Inhibition of protein synthesis augments the heme-hemopexin-mediated accumulation of MT-1 mRNA. Activation of heme oxygenase (HO) gene transcription by heme requires the synthesis of one (or more) heme-inducible proteins that are labile or become labile upon cycloheximide-sensitive processing or activation. Our comparison of MT and HO points to significant differences in the mechanisms of gene regulation by heme. The concomitant regulation of gene expression of MT-1 and HO in response to heme-hemopexin appears to be a concerted adaptive response of the cells, mediated at the level of the plasma membrane hemopexin receptor, and may relate to the proposed role of MT as an intracellular antioxidant or to a need to sequester zinc which otherwise would compete with iron and occupy sites on regulatory proteins such as the iron-responsive elements.

Alcedo, J. A., et al. (1994). "The genotoxic carcinogen chromium(VI) alters the metal-inducible expression but not the basal expression of the metallothionein gene in vivo." Carcinogenesis 15(5): 1089-1092.

The ability of the carcinogen chromium(VI) to affect the basal and zinc-inducible expression of liver metallothionein was examined in 14- and 18-day chicken embryos in vivo. Metallothionein expression varied with the stage of embryo development, with basal steady-state mRNA levels being approximately three times lower in livers of 18-day versus 14-day chicken embryos. Chromium(VI) treatment had no effect on the basal steady-state levels of metallothionein mRNA and protein in either 14- or 18-day chicken embryo liver. Treatment of 14-day embryos with zinc(II) resulted in a 3- to 5-fold increase in steady-state levels of metallothionein mRNA in liver. Pre-treatment of 14-day embryos with chromium(VI) inhibited the zinc(II)-induced increase in steady-state levels of metallothionein mRNA and protein in liver by 30-50%. In contrast, chromium(VI) and/or zinc(II) treatments had no effect on steady-state levels of beta-actin mRNA.

Alhonen, L., et al. (1996). "Transgenic mice expressing the human ornithine decarboxylase gene under the control of mouse metallothionein I promoter." Biochem J 314 (Pt 2): 405-408.

We have generated a transgenic mouse line harbouring the human ornithine decarboxylase gene under the control of mouse metallothionein I promoter. Even in the absence of an exposure to heavy metals, ornithine decarboxylase was over-expressed in heart, testis, brain, and especially in liver, of the transgenic animals. An exposure of the transgenic mice to zinc further enhanced the enzyme activity to a level which in liver represented up to 8000-fold increase in comparison with non-transgenic animals. The striking stimulation of liver ornithine decarboxylase activity upon treatment of the transgenic mice with zinc was accompanied by a nearly 150-fold increase in the hepatic putrescine content as compared with similarly treated non-transgenic animals. Even though the liver putrescine concentration reached that of spermidine and spermine in the transgenic animals, the contents of the higher polyamines only transiently increased upon zinc administration and then returned to the basal level. These findings once again indicate that mammalian cells possess extremely powerful regulatory machinery to prevent an over-accumulation of spermidine and spermine in non-dividing cells, and that very high tissue putrescine concentrations can be tolerated, at least for periods of a few days, with seemingly no phenotypic changes.

Allard, S., et al. (1995). "Recombination of endogenous D2 dopamine receptor gene with a metallothionein promoter in GH4C1 cells confers functional and inducible D2 response." Biochim Biophys Acta 1260(1): 43-48.

We have previously shown that expression of a functional endogenous D2 short dopamine receptor is obtained in GH4C1 cells following transfection with a plasmid that confers resistance to neomycin (pRSVNeo) (Allard et al. (1993) Biochem. Biophys. Res. Commun. 193, 801-807). In order to better understand the mechanisms responsible for such a phenomenon, we cloned and sequenced the 5' region of the D2 gene present in native GH4C1 cells as well as the cDNA of transfected cells. No homology with the published sequence of the rat D2 dopamine receptor promoter

was found; however, this region has perfect homology with the mouse metallothionein promoter. In cells expressing D2 receptor, the promoter is fully functional and can regulate dopaminergic D2 receptor mRNA levels and receptor expression in a dose-dependent manner in the presence of Zn2+ or Cd2+. The receptor level is raised from 500 to 3000 fmol/mg of protein in the presence of 100 microM of Zn2+. These results suggest that in GH4C1 cells, a recombination between the mouse metallothionein promoter and the D2 dopamine receptor took place. This system provides us with a cell line expressing an endogenous dopamine D2 receptor in which the level of expression can be easily modulated.

Andersen, R. D., et al. (1986). "Rat metallothionein-1 structural gene and three pseudogenes, one of which contains 5'-regulatory sequences." Mol Cell Biol 6(1): 302-314.

As shown by Southern blot analysis, the metallothionein-1 (MT-1) genes in rats comprise a multigene family. We present the sequence of the MT-1 structural gene and compare its features with other metallothionein genes. Three MT-1 pseudogenes which we sequenced apparently arose by reverse transcription of processed mRNA transcripts. Two of these, MT-1 psi a and MT-1 psi c, are retrogenes which derive from the MT-1 mRNA, having diverged from the MT-1 gene 6.9 and 2.6 million years ago, respectively. The third, MT-1 psi b, differs from the MT-1 cDNA by only three nucleotide alterations. Surprisingly, MT-1 psi b also preserves sequence homology for 142 base pairs 5' to the transcription initiation site of the parent gene; it contains a promoter sequence sufficient for specifying metal ion induction. We identified, by S1 nuclease mapping, an RNA polymerase II initiation site 432 base pairs 5' of the MT-1 transcription initiation site of the MT-1 structural gene which could explain the formation of the mRNA precursor to this pseudogene. We were unable to detect MT-1 psi b transcripts, either in liver tissue or after transfection. We conclude that the absence of detectable transcripts from this pseudogene is due to either a reduced level of transcription or the formation of unstable transcripts as a consequence of the lack of a consensus sequence normally found 3' of transcription termination in the MT-1 structural gene.

Andersen, R. D., et al. (1987). "Metal-dependent binding of a factor in vivo to the metal-responsive elements of the metallothionein 1 gene promoter." Mol Cell Biol 7(10): 3574-3581.

Using the technique of genomic footprinting, we demonstrate cadmium-inducible protection from dimethyl sulfate (DMS) modification of guanine residues in vivo in five metal-responsive elements (MREs) in the promoter of the rat metallothionein 1 (MT-1) gene. We also identify a site of extreme DMS hyperreactivity which, like the MRE protection, occurs only after metal ion induction. With this hyperreactive site as an indicator, we can measure the kinetics of induction and deinduction. Changes in the intracellular metal ion concentrations are reflected in alterations in the reactivity with DMS of guanine residues in the MT-1 gene promoter. Lastly, for both control and metal-induced cells, we observe DMS protection and enhancement of a binding site (located 5' of the distal MRE) which is a consensus sequence for the Sp1 transcription factor. Transfection experiments with deletion mutations of a fusion gene construct indicate both that a sequence region which includes this GC box regulates the basal level of expression of the MT-1 gene and that increasing the number of MREs in the promoter increases the induced level of transcription. Our genomic footprinting and transfection data together suggest that (i) a transcription factor, possibly Sp1, plays an important role in regulating the basal level of expression of the MT-1 gene and (ii) metal induction involves the metal-dependent binding to a sequence-specific binding factor which responds to changes in intracellular metal ion levels.

Andrews, G. K. (1990). "Regulation of metallothionein gene expression." Prog Food Nutr Sci 14(2-3): 193-258.

The metallothioneins are small, cysteine-rich proteins that have the capacity for high affinity binding of heavy metal ions, and whose synthesis is regulated by metal ion concentrations. These properties suggest that they play pivotal roles in the metabolism of the relatively nontoxic essential metals (zinc and copper), as well as toxic heavy metals (cadmium), a concept supported by a variety of studies of cells in culture, as well as in intact animals. Expression of the metallothionein genes may have important implications in the nutritional status of the animal, in its response to stresses (inflammation, heavy metal toxicity), and in embryonic, fetal and neonatal development. The complementary DNAs and genes that encode the metallothioneins have been cloned and analyzed from a wide variety of eukaryotes. Striking features of the metallothioneins include: their high degree of amino acid sequence similarity (including conservation in the placement of cysteine residues in the molecule reflecting their function in metal binding); a conserved tripartite gene structure; and their transcriptional induction by metal ions, as well as other hormonal and environmental stimuli. The precise mechanisms and biochemical pathways by which cells transduce environmental signals into transcriptional induction of the metallothionein genes are beginning to be defined. Recent studies indicate that metal effects are exerted via positive trans-acting factors induced to interact with cis-acting DNA sequences in the promoter, in turn leading to transcriptional induction. However, the metallothionein gene promoter is structurally complex,

and contains binding sites for a variety of nuclear proteins that likely regulate basal as well as induced levels of expression of these genes. Recent studies also suggest the possible involvement of post-transcriptional processes in the regulation of metallothionein levels in the cell. Furthermore, evidence of striking differences in the levels of metallothionein gene expression among various cell types in vivo have recently been documented. Although several detailed reviews of the metallothioneins have been published recently, this review will focus, in large part, on the molecular biology of the metallothioneins, with particular emphasis on recent advances in our understanding of the mechanisms regulating expression of these interesting and important genes. Given the large volume of literature on the metallothioneins and the space limitations of this review, it is impossible to comprehensively cite the studies of each of my colleagues who have contributed so much to this field. Instead the reader is often directed to reviews of this subject for much of the earlier literature, and emphasis is placed on more current publications in this field.

Andrews, G. K. (2000). "Regulation of metallothionein gene expression by oxidative stress and metal ions." Biochem Pharmacol 59(1): 95-104.

The metallothioneins (MT) are small, cysteine-rich heavy metal-binding proteins which participate in an array of protective stress responses. Although a single essential function of MT has not been demonstrated, MT of higher eukaryotes evolved as a mechanism to regulate zinc levels and distribution within cells and organisms. These proteins can also protect against some toxic metals and oxidative stress-inducing agents. In mice, among the four known MT genes, the MT-I and -II genes are most widely expressed. Transcription of these genes is rapidly and dramatically up-regulated in response to zinc and cadmium, as well as in response to agents which cause oxidative stress and/or inflammation. The six zinc-finger metal-responsive transcription factor MTF-1 plays a central role in transcriptional activation of the MT-I gene in response to metals and oxidative stress. Mutation of the MTF-1 gene abolishes these responses, and MTF-1 is induced to bind to the metal response elements in proximal MT promoter in cells treated with zinc or during oxidative stress. The exact molecular mechanisms of action of MTF-1 are not fully understood. Our studies suggest that the DNA-binding activity of MTF-1 in vivo and in vitro is reversibly activated by zinc interactions with the zinc-finger domain. This reflects heterogeneity in the structure and function of the six zinc fingers. We hypothesize that MTF-1 functions as a sensor of free zinc pools in the cell. Changes in free zinc may occur in response to chemically diverse inducers. MTF-1 also exerts effects on MT-I gene transcription which are independent of a large increase in MTF-1 DNA-binding activity. For example, cadmium, which has little effect on the DNA-binding activity of MTF-1 in vivo or in vitro, is a more potent inducer of MT gene expression than is zinc. The basic helix-loop-helix-leucine zipper protein, USF (upstream stimulatory factor family), also plays a role in regulating transcription of the mouse MT-I gene in response to cadmium or H2O2. Expression of dominant negative USF-1 or deletion of its binding site from the proximal promoter attenuates induction of the mouse MT-I gene. USF apparently functions in this context by interacting with as yet unidentified proteins which bind to an antioxidant response element which overlaps the USF-binding site (USF/ARE). Interestingly, this composite element does not participate in the induction of MT-I gene transcription by zinc or redox-cycling quinones. Thus, regulation of the mouse MT-I gene by metals and oxidative stress involves multiple signaling pathways which depend on the species of metal ion and the nature of the oxidative stress.

Andrews, G. K. and E. D. Adamson (1987). "Butyrate selectively activates the metallothionein gene in teratocarcinoma cells and induces hypersensitivity to metal induction." Nucleic Acids Res 15(13): 5461-5475.

The expression of metallothionein genes (MT-I and MT-II) was shown to be enhanced within 2 h of addition of 2.5-5 mM sodium butyrate to cultures of teratocarcinoma cells. Both undifferentiated stem cells (F9 and OC15) and differentiated cells (PSA5E and OC15 END) reacted similarly to butyrate by increased accumulation of MT mRNAs. As expected, all of the teratocarcinoma cells that were tested also responded to Zn2+ and Cd2+ by 5- to 10-fold increases in MT mRNA accumulation within 2-24 h of metal addition to the culture media. Surprisingly, MT genes in cells pretreated with butyrate were hypersensitive to metal induction, and this was demonstrated by accumulated transcript levels and by synthesis of MT protein. The maximal metal response was obtained by exposure of cells to butyrate for around 5-8 h together with 10 microM heavy metals. Metal additions to culture media over a range of concentrations and times only induced half the levels of MT mRNA that were achieved by butyrate plus metals. Butyrate enhanced the rate of accumulation of MT mRNA in response to metals, increased the sensitivity of the MT gene to metals, and protected cells from toxic effects of high concentrations of metals. The butyrate and metal ion responses were selective in that no accumulation of c-myc, c-fms, HSP-70, or AFP mRNA was detected. However, c-fos mRNA accumulated in cells exposed to toxic concentrations of metals (50 microM and higher) and this was also potentiated by butyrate treatment. These results suggest that butyrate alters the chromatin conformation of both the MT-I and MT-II genes leading to an accentuated transcriptional response to

metals.

Andrews, G. K., et al. (1984). "The ontogeny of expression of murine metallothionein: comparison with the alpha-fetoprotein gene." Dev Biol 103(2): 294-303.

The ontogeny of expression of mouse metallothionein was studied by RNA dot and Northern blot hybridization using a cloned cDNA probe. In some instances the synthesis of metallothionein was analyzed by cell-free translation of RNA as well as pulse-labeling of proteins in short-term organ cultures followed by polyacrylamide gel electrophoresis. Interesting parallels between metallothionein and alpha-fetoprotein gene expression during development were noted. Like alpha-fetoprotein mRNA (Dziadek and Andrews, 1983), metallothionein mRNA was found to be abundant in developing liver as well as in visceral yolk sac endoderm. In addition, metallothionein mRNA was abundant in parietal yolk sac. During liver development metallothionein and alpha-fetoprotein mRNAs were abundant by Day 12 of gestation, increasing to maximal levels on Day 16 and decreasing during late fetal and neonatal life to basal levels in adult. Metallothionein mRNA increased in maternal liver and was also abundant in certain hepatomas. Synthesis of metallothionein and levels of metallothionein mRNA in visceral yolk sac increased from Day 9 of gestation to maximal levels on Days 11-12 and then decreased abruptly after Day 15. RNA from differentiated teratocarcinoma cells with primitive, parietal or visceral endoderm characteristics each contained high levels of metallothionein mRNA, whereas, levels of this mRNA varied widely among embryonal carcinoma stem cell lines. alpha-Fetoprotein mRNA was not detected in embryonal carcinoma cells but was expressed in visceral endoderm-like differentiated cells. These results indicate that parietal and visceral endoderm cells actively express the metallothionein gene and further suggest that expression may be initiated at the earlier stage of primitive endoderm.

Andrews, G. K., et al. (1987). "Metallothionein gene regulation in the preimplantation rabbit blastocyst." Development 100(3): 463-469.

Expression of metallothionein (MT) genes in the preimplantation rabbit blastocyst was analysed by determination of the levels of MT mRNA and relative rates of MT synthesis. MT was found to be constitutively expressed at low levels in the blastocyst. Exposure of the day-6 blastocyst to zinc ions in vitro rapidly increased the level of MT gene expression in a dose-dependent manner, with a ten-fold induction in the relative rate of synthesis at 400 microM-Zn2+. Ion-exchange chromatography of pulse-labelled blastocyst protein showed that the relative rates of synthesis of both MT-I and MT-II were markedly increased following zinc treatment, with MT-I being the predominant isometallothionein. Zinc induction of MT synthesis in the blastocyst was also detected on day 4 of gestation just after the morula-to-blastocyst transition. In contrast to the zinc effects on MT, in vitro exposure to 10 microM-Cd2+ resulted in a large induction of MT mRNA but only a modest increase in the relative rate of MT synthesis. Cadmium was found to be toxic to the day-6 blastocyst, and 10 microM-Cd2+ induced an acute stress response as indicated by a dramatic induction of heat-shock protein (HSP-70) gene expression.

Andrews, G. K., et al. (1991). "Metallothionein gene expression and metal regulation during preimplantation mouse embryo development (MT mRNA during early development)." Dev Biol 145(1): 13-27.

In order to provide information concerning gene expression and regulation in the preimplantation mammalian embryo, and to explore the roles of metallothionein (MT) during this period of development, the constitutive and metal-induced MT mRNA levels in mouse ova, preimplantation embryos, and oviducts were determined. These results were correlated with the effects of transient exposure to high levels of metals (zinc (Zn) or cadmium (Cd] on the continued development of preimplantation embryos into blastocysts in culture. RNA from preimplantation mouse embryos at different stages of development (Days 1 through 4 of gestation; D1 = vaginal plug) was analyzed using the reverse transcriptase-polymerase chain reaction (RT-PCR) to specifically amplify MT-I and MT-II mRNA transcripts. MT-I mRNA in ova, preimplantation embryos, and oviducts was detected using in situ hybridization. This mRNA in the oviduct was also analyzed by Northern blotting. The results establish that the mouse MT genes are coordinately and constitutively expressed at low basal levels in ova and preimplantation mouse embryos. In unfertilized (ova), fertilized (one-cell) eggs, and two-cell embryos, the MT-I gene was not detectably responsive to metal ions, whereas in later cleavage stage embryos (four- and eight-cell) the MT-I gene was detectably responsive to metals in some blastomeres of some of the embryos. In contrast, after the third cleavage this gene was highly metal-inducible in essentially all cells of the embryo (morula/blastocyst). Surprisingly, the appearance of metal responsiveness of the MT genes during development correlated with decreased Zn toxicity and increased Cd toxicity; two-cell embryos were Zn-sensitive and Cd-resistant, whereas eight-cell and older embryos were Zn-resistant and Cd-sensitive. In the oviduct, MT-I mRNA was not abundant in total RNA, but was detected specifically in the epithelial cells of the isthmus region and was elevated in these cells on D3 and D4 of gestation. In the oviduct, only isthmus epithelial cells responded to metals (Zn or Cd) by increased accumulation of this mRNA. These studies

suggest that preimplantation mouse embryo develops the capacity to respond to metals in the environmental milieu by induction of MT gene expression at about the third cleavage. Whether the lack of responsiveness of these genes before this stage reflects transcriptional repression or attenuated metal ion influx and/or enhanced efflux remains to be determined. Sensitivity and resistance of preimplantation embryos to acute metal toxicity involve mechanisms other than MT gene expression in preimplantation mouse embryos.(ABSTRACT TRUNCATED AT 400 WORDS)

Armendariz, A. D., et al. (2006). "Gene expression profiling in wild-type and metallothionein mutant fibroblast cell lines." Biol Res 39(1): 125-142.

The role of metallothioneins (MT) in copper homeostasis is of great interest, as it appears to be partially responsible for the regulation of intracellular copper levels during adaptation to extracellular excess of the metal. To further investigate a possible role of MTs in copper metabolism, a genomics approach was utilized to evaluate the role of MT on gene expression. Microarray analysis was used to examine the effects of copper overload in fibroblast cells from normal and MT I and II double knock-out mice (MT-/-). As a first step, we compared genes that were significantly upregulated in wild-type and MT-/- cells exposed to copper. Even though wild-type and mutant cells are undistinguishable in terms of their morphological features and rates of growth, our results show that MT-/- cells do not respond with induction of typical markers of cellular stress under copper excess conditions, as observed in the wild-type cell line, suggesting that the transcription initiation rate or the mRNA stability of stress genes is affected when there is an alteration in the copper store capacity. The functional classification of other up-regulated genes in both cell lines indicates that a large proportion (>80%) belong to two major categories: 1) metabolism; and 2) cellular physiological processes, suggesting that at the transcriptional level copper overload induces the expression of genes associated with diverse molecular functions. These results open the possibility to understand how copper homeostasis is being coordinated with other metabolic pathways.

Averbeck, N. B., et al. (2001). "Molecular control of copper homeostasis in filamentous fungi: increased expression of a metallothionein gene during aging of Podospora anserina." Mol Gen Genet 264(5): 604-612.

The lifespan of the ascomycete Podospora anserina was previously demonstrated to be significantly increased in a copper-uptake mutant, suggesting that copper is a potential stressor involved in degenerative processes. In order to determine whether changes in copper stress occur in the cells during normal aging of cultures, we cloned and characterized a gene coding for a component of the molecular machinery involved in the control of copper homeostasis. This gene, PaMt1, is a single-copy gene that encodes a metallothionein of 26 amino acids. The coding sequence of PaMt1 is interrupted by a single intron. The deduced amino acid sequence shows a high degree of sequence identity to metallothioneins of the filamentous ascomycete Neurospora crassa and the basidiomycete Agaricus bisporus, and to the N-terminal portion of mammalian metallothioneins. Levels of PaMt1 transcript increase in response to elevated amounts of copper in the growth medium and during aging of wild-type cultures. In contrast, in the long-lived mutant grisea, transcript levels first increase but then decrease again. The ability of wild-type cultures to respond to exogenous copper stress via the induction of PaMt1 transcription is not affected as they grow older.

Aydemir, T. B., et al. (2006). "Zinc supplementation of young men alters metallothionein, zinc transporter, and cytokine gene expression in leukocyte populations." Proc Natl Acad Sci U S A 103(6): 1699-1704.

An effective measure to assess zinc status of humans has remained elusive, in contrast to iron, where a number of indicators of metabolism/function are available. Using monocytes, T lymphocytes, and granulocytes isolated by magnetic sorting and dried blood spots (DBS) derived from 50 mul of peripheral blood, we evaluated the response of metallothionein (MT), zinc transporter, and cytokine genes to a modest (15 mg of Zn per day) dietary zinc supplement in human subjects. Transcript abundance was measured by quantitative real-time RT-PCR (QRT-PCR). Zinc supplementation increased MT mRNA abundance by up to 2-fold in RNA from leukocyte subsets, and 4-fold in RNA from DBS. Transcript levels for the zinc transporter genes ZnT1 and Zip3 were increased and decreased, respectively, by zinc supplementation. Expression of the ZnT and Zip genes among leukocyte subsets differ by up to 270-fold. Monocytes and granulocytes from supplemented subjects were activated by LPS, whereas T lymphocytes were activated by mimicking antigen presentation. With zinc consumption, TNF-alpha and IL-1beta expression was greater in activated monocytes and granulocytes, and IFN-gamma mRNA levels were higher in activated T lymphocytes. These studies show that QRT-PCR is a tool to reliably measure transcript abundance for nutritionally responsive genes in human subjects, and that a small sample of whole dried blood, when appropriately collected, can be used as the source of total RNA for QRT-PCR analysis. The results obtained also show that zinc supplementation of human subjects programs specific leukocytic subsets to show enhanced cytokine expression upon activation by stimulators of immunity.

Bacolla, A. and F. Y. Wu (1991). "Mung bean nuclease

cleavage pattern at a polypurine.polypyrimidine sequence upstream from the mouse metallothionein-I gene." Nucleic Acids Res 19(7): 1639-1647.

Mung bean nuclease, an enzyme specific for single-stranded DNA, was used to probe a non-B DNA structure present in the mouse metallothionein-I gene. The region sensitive to the enzyme was constituted by a 128 base-pair long polypurine.polypyrimidine sequence located at 1.2-kb from the start of transcription. A detailed analysis of the mung bean nuclease cleavage pattern revealed that: (i) under conditions of supercoiling and low pH a triplex structure was formed, (ii) the triplex was flanked by a sequence with the potential of forming a Z-DNA structure, (iii) most of the enzymatic activity was localized at some of the junctions between double-stranded and triple-stranded DNA and at mismatches in the triplex, (iv) no unpaired bases were observed in the loop or outside the triplex, and (v) the triplex was present in more than one configuration.

Bai, G., et al. (1993). "Combinatorial regulation by promoter and intron 1 regions of the metallothionein SpMTA gene in the sea urchin embryo." Mol Cell Biol 13(2): 993-1001.

The SpMTA metallothionein gene of the sea urchin Strongylocentrotus purpuratus is regulated developmentally, histospecifically, and by heavy-metal induction. The sequenced 5' flank of the gene can be divided into proximal, middle, and distal regions, each containing a pair of metal response elements (MREs). Canonical 7-bp core sequences are present in all except the middle-region MREs c and d, which contain 1-bp mismatches. Metal-induced expression in transgenic blastulae was increased with each consecutive addition of the middle and distal regions to a chimeric reporter gene construct containing the proximal SpMTA promoter region. Reduced metal induction through point mutation of the distal MREs e and f indicated that the MREs themselves were largely responsible for the transcriptional increase. These activities were further enhanced by SpMTA intron 1, but not when a specific interior region of the intron had been deleted. The atypical MREs c and d did not support induction by themselves, i.e., when present alone with mutated proximal MREs a and b. However, in the presence of intron 1, they were able to substitute for the nullified MREs a and b in the promotion of metal-induced expression. This capability suggests, furthermore, that these atypical MREs, in addition to responding to an intron 1 region, participate cooperatively with the canonical proximal MREs.

Ban, Q. Y., et al. (2008). "[Cloning and expression of a novel metallothionein gene LbMT2 from Limonium bicolor]." Yi Chuan 30(8): 1075-1082.

The full length cDNA of a novel metallothionein (LbMT2) gene was cloned from a cDNA library of Limonium bicolor. The LbMT2 gene cloned is 518 bp in length, which includes a 64 bp of 5' untranslated region (UTR) and a 205 bp of 3' untranslated region. This gene has an open reading frame (ORF) of 249 bp in length, encoding a protein of 82 amino acid residues with the molecular mass of 8.1 kDa and theoretical pI of 4.71. The expression of LbMT2 gene in L. bicolor in response to CuSO4, CdCl2, NaCl, cold, and PEG was further investigated using real time quantitative PCR. In both leaf and root of L. bicolor, the expression of LbMT2 was induced by CuSO4, CdCl2, NaCl, and cold, but inhibited by PEG stress. LbMT2 gene was inserted into a prokaryotic expression vector (pGEX-4T-2) to produce the recombinant expression vector pGEX-LbMT2. The expression of LbMT2 in E. coli BL21 was induced with IPTG, which produced a protein band with expected size of 35 kDa on SDS-PAGE.

Banday, U. Z., et al. (2020). "Heavy metal toxicity has an immunomodulatory effect on metallothionein and glutathione peroxidase gene expression in Cyprinus carpio inhabiting a wetland lake and a culture pond." Chemosphere 251: 126311.

The study provides cumulative data on the status of the two water bodies. The study designed revealed physicochemical properties (temperature, dissolved oxygen, pH, total dissolved solids and conductivity) to be in the desirable range, however, amongst the heavy metals excepting for Cd all were found to be higher than the permissible limits set by WHO and USEPA. It was observed that these elements cast their impact on bioindices (hepatosomatic index, condition factor, spleenosomatic index and kidney somatic index), renal marker enzyme (creatine kinase), hepatic marker enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase), histology of immune organs (liver, spleen, head-kidney and thymus) and level of serum immunoglobulin (IgM). Further, expression levels of Metallothionein (MT) and Glutathione peroxidase (GPX) genes in immune-related tissues (liver, spleen, head-kidney, thymus and blood) observed indicates metal pollution and abiotic stresses. These alterations are reliable indicators of the cellular and humoral immune response in Cyprinus carpio.

Banni, M., et al. (2010). "Metallothionein gene expression in liver of rats exposed to cadmium and supplemented with zinc and selenium." Arch Environ Contam Toxicol 59(3): 513-519.

Cadmium (Cd), one of the most widely distributed heavy metals, is highly toxic to humans and animals. It is well known that zinc (Zn) and selenium (Se) administration reduce the Cd-induced toxicity and that metallothioneins can have a protective effect to mitigate Cd toxicity in biological systems. In this study we report the expression analysis of the two

metallothioneines gene classes MT-1 and MT-2 as well as the total metalloprotein content in the liver of rats exposed to Cd (200 ppm), Cd + Zn (200 ppm + 500 ppm), Cd + Se (200 ppm + 0.1 ppm) or Cd + Zn + Se (200 ppm + 500 ppm + 0.1 ppm) in their drinking water for 35 days. Metals accumulation was quantified in rat liver. Cd decreased significantly the hepatic concentrations of Se and increased those of Zn. The treatment of Cd-exposed rats with Se alone or combined with Zn reversed the Cd-induced depletion of Se concentrations in the liver. However, Zn or Zn + Se administration significantly increased the liver Cd uptake and had no effect on the Cd-induced increase in hepatic concentrations of Zn. The molecular assay showed a decreasing trend of MT-1 relative gene expression levels in animals supplemented with Zn (6.87-fold), Se (3.58-fold), and their combination (1.69-fold) when compared to Cd-treated animals (16.22-fold). Upregulation of the MT-2 expression were recorded in all conditions, although fold induction levels were less pronounced than MT-1 expressions. Our data suggest that the well-established protective effect of Zn and Se against Cd-induced toxicity passes through non-MT gene expression mechanisms, being more dependent on the oxidative stress status of the cell.

Bauknecht, T., et al. (1993). "Gene structure and expression analysis of the epidermal growth factor receptor, transforming growth factor-alpha, myc, jun, and metallothionein in human ovarian carcinomas. Classification of malignant phenotypes." Cancer 71(2): 419-429.

This study reports the structure and expression rates of genes of the transforming growth factor-alpha (TGF-alpha) signal transduction pathway (TGF-alpha, epidermal growth factor receptor [EGF-R], jun, myc, and metallothionein [MT]) in 47 specimens of ovarian cancer and 21 nonmalignant tissues. The objective was to establish a direct correlation between the genetic activities and the malignant phenotype of the ovarian cancer. The Southern blot technique identified four samples with myc amplification and two with rearranged EGF-R genes. By using the S1 nuclease assay, the analysis of myc transcription showed a similar use of both promotors. Although the size of the investigated transcripts was unaltered, significant differences in the transcription rates were noticed in malignant tissue probes (using northern blot analysis and RNAase protection assay). The following results of messenger RNA analysis in ovarian cancer were observed: EGF-R, negative in 25%, low in 65%, and strongly positive in 10%; TGF-alpha, negative in 34%, low in 36%, and strongly positive in 30%; myc, negative in 8%, low in 64%, and strongly positive in 28%; jun, negative in 4%, low in 58%, and strong in 38%; and MT, low in 80% and strongly positive in 20%. In most nonmalignant tissues studied, no or only a low expression of TGF-alpha, EGF-R, and myc. was found. A comparison of these messenger RNA results with the clinical data from tumors showed four different subgroups of ovarian carcinomas. The results of chemotherapy were known in 32 cases. Tumors with negative or low expression rates of all investigated genes did not respond to chemotherapy; 13 of 18 tumors with high expression rates did respond. Additional signal transduction chains distinct from the TGF-alpha pathway, however, are likely to influence both the expression and activity of transcription factors and MT.

Baurand, P. E., et al. (2016). "Metallothionein gene expression in embryos of the terrestrial snail (Cantareus aspersus) exposed to cadmium and copper in the Bordeaux mixture." Environ Sci Pollut Res Int 23(4): 3068-3072.

The response specificity of three metallothionein (MT) genes (CdMT, CuMT and Cd/CuMT) was assessed after long-term exposure (20 days) of Cantareus aspersus eggs to cadmium (Cd) (2 to 6 mg/L) or to the fungicide Bordeaux mixture (BM) (2.5 and 7.5 g/L). MT gene expression measured by quantitative real-time PCR (qRT-PCR) revealed that in the unexposed embryos, the transcript levels of the three MT genes decreased significantly through embryonic development. However, the CdMT gene was strongly upregulated with increasing Cd exposure concentration, whereas the transcript levels of the other two genes increased less pronouncedly, but significantly above an exposure concentration of 4 mg Cd/L. Upon exposure to BM, all three MT genes were significantly upregulated above a BM concentration of 2.5 g/L. It is concluded that long-term Cd exposure in hatched snails induced patterns of MT gene expression that differed from those obtained after short-term exposure (24 h).

Baurand, P. E., et al. (2016). "Differential sensitivity of snail embryos to cadmium: relation to age and metallothionein gene expression." Environ Sci Pollut Res Int 23(4): 3062-3067.

The aim of this study was to determine whether cadmium (Cd) sensitivity of Cantareus aspersus embryos is age-dependent and influenced by metallothionein (MT) gene expression. Hatching success and the expression of three MT isoform genes (Ca-CdMT, Ca-CuMT and Ca-Cd/CuMT) were measured in embryos exposed to increasing Cd concentrations for 24 h starting on the sixth day of development. Isoform gene expression was quantified on days 7 and 12 after exposure. Results were compared to those of embryos exposed to the same conditions as above, but from the beginning of embryogenesis (day 0). Transcription of the Cd-specific MT gene (Ca-CdMT) was observed from the

first day of development, whereas the two other genes did not respond to Cd at all. Overall, Cd sensitivity of embryos decreased with increasing age of development, as assessed by age-dependent increase of EC50 values for hatching rate, and increasing Cd threshold concentrations for Ca-CdMT expression.

Beach, L. R. and R. D. Palmiter (1981). "Amplification of the metallothionein-I gene in cadmium-resistant mouse cells." Proc Natl Acad Sci U S A 78(4): 2110-2114.

Friend leukemia cells resistant to cadmium toxicity were selected. More than 70% of total cysteine incorporation in these cells was into the metal-binding protein, metallothionein. We used cDNA and genomic DNA clones containing the metallothionein-I gene to measure the concentration of its mRNA, the rate of gene transcription, and the number of genes. On a per cell basis, optimally induced, cadmium-resistant cells have a 14-fold more metallothionein-I mRNA, a 6-fold higher rate of metallothionein-I gene transcription, and 6-fold more metallothionein-I genes than do nonresistant cells. Metaphase spreads revealed that the resistant cells are nearly tetraploid and contain, on the average, three very small chromosomes that are absent from non-resistant Friend cells.

Beattie, J. H., et al. (1996). "Cold-induced expression of the metallothionein-1 gene in brown adipose tissue of rats." Am J Physiol 270(5 Pt 2): R971-977.

Heat production by brown adipose tissue (BAT) is important for thermoregulation in a cold environment. During thermogenesis, oxygen utilization increases, with an associated rise in free radical generation. Our objective was to investigate the expression of metallothionein (MT), which is thought to have an antioxidant role in BAT of rates transferred from 25 to 6 degrees C for 6 or 24 h or maintained at 25 degrees C throughout the study (control group). For comparison, MT expression was also measured in white adipose tissue (WAT), liver, and kidney. MT-1 mRNA and 18S rRNA were measured by Northern blotting using specific digoxigenin-labeled antisense oligonucleotide probes with chemiluminescence detection, and MT-1 protein was determined by radioimmunoassay. MT-1 mRNA in BAT increased after 6 h, and the mRNA level after 24 h was equivalent to that in liver 6 h after injection of rats with 10 mg Zn/kg. By 24 h, liver and kidney MT-1 protein had increased relative to the controls by 3- and 1.4-fold, respectively, but in BAT the relative induction was 16-fold. Zn injection did not affect BAT MT-1. As with MT-1 protein, Zn in BAT increased only after 24-h cold exposure. WAT MT-1 was not affected by any treatment. It is concluded that could exposure induces MT-1 in BAT, but in contrast to other tissues induction may be independent of Zn.

Bhave, M. R., et al. (1988). "Methylation status and organization of the metallothionein-I gene in livers and testes of strains of mice resistant and susceptible to cadmium." Toxicology 50(3): 231-245.

The methylation status, copy number and organization of the metallothionein-I (MT-I) gene was studied in hepatic and testicular DNAs of mouse strains resistant (BALB/c) and susceptible (NFS) to cadmium-induced testicular toxicity. Digestion of DNAs by the restriction enzymes BamHI, EcoRI and HindIII produced identical patterns for hepatic and testicular DNAs of both strains, indicating that there was no apparent difference in the gross genomic organization or in copy number of the MT-I gene in the 2 types of tissues from either strain. Digestion with MspI, HpaII, AvaII and HhaI indicated that the hepatic DNAs of both strains were under-methylated as compared to the testicular DNAs. However, the NFS DNAs lacked a fragment that was consistently observed in the MspI digests of BALB/c DNAs, suggesting the presence of a polymorphic CCGG site. This site was localized by double digestion of DNAs with BstEII or HindIII and MspI to the 3' end of the MT-I gene. Differences in methylation status may account for the differential susceptibility of the 2 tissues to cadmium toxicity. The higher degree of MT-I gene methylation may result in slower or inefficient induction of MT in the testes, resulting in greater sensitivity to metal toxicity in testes than in liver. However, differences in methylation status alone do not seem to account for the interstrain differences in cadmium toxicity, and other factors, such as differences in genetic organization, seem to be involved in the inducibility of MT-I gene in different strains.

Bi, Y., et al. (2006). "Superinduction of metallothionein I by inhibition of protein synthesis: role of a labile repressor in MTF-1 mediated gene transcription." J Biochem Mol Toxicol 20(2): 57-68.

Induction of metallothioneins (MTs) through the metal-activated transcription factor-1 (MTF-1) provides a model response for analyzing transcriptional gene regulation by heavy metals. Here, we report inhibition of protein synthesis by cycloheximide (CHX) increases induction of Mt1 by approximately five-fold, a phenomenon designated as "superinduction." Characterization of superinduction revealed it is time- and concentration-dependent of CHX, requires the presence of an MTF-1 activator, and occurs at a transcriptional level, suggesting a labile repressor in the control of Mt1 induction. Genetic analyses using Mtf1 null cells and a metal response element (MRE)-driven reporter construct showed that superinduction of Mt1 is mediated through MTF-1 and MRE-dependent transcription. Analyses of intracellular zinc content by inductively coupled plasma emission spectroscopy and fluorescence imaging demonstrated that treatment with CHX alone or CHX plus an inducer does not increase

the total zinc accumulation or the concentration of free zinc in cells under the conditions in which superinduction occurs. Moreover, superinduction was observed in cells cultured in a zinc-depleted medium, suggesting that superinduction does not involve elevation of intracellular zinc concentration. Northern blotting showed that Cd, CHX, or Cd + CHX does not affect the expression of the mRNA of MTF-1. Immunoblotting using antibodies specific for MTF-1 demonstrated that Cd induces a down-regulation of the MTF-1 protein, whereas cotreatment with Cd and CHX blocked the Cd-induced degradation of MTF-1. The findings reveal a new mechanistic aspect of the superinduction of Mt1, in which a labile repressor negatively controls agonist-induced turnover of the MTF-1 protein.

Bilecen, K., et al. (2005). "Triticum durum metallothionein. Isolation of the gene and structural characterization of the protein using solution scattering and molecular modeling." J Biol Chem 280(14): 13701-13711.

A novel gene sequence, with two exons and one intron, encoding a metallothionein (MT) has been identified in durum wheat Triticum durum cv. Balcali85 genomic DNA. Multiple alignment analyses on the cDNA and the translated protein sequences showed that T. durum MT (dMT) can be classified as a type 1 MT. dMT has three Cys-X-Cys motifs in each of the N- and C-terminal domains and a 42-residue-long hinge region devoid of cysteines. dMT was overexpressed in Escherichia coli as a fusion protein (GSTdMT), and bacteria expressing the fusion protein showed increased tolerance to cadmium in the growth medium compared with controls. Purified GSTdMT was characterized by SDS- and native-PAGE, size exclusion chromatography, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. It was shown that the recombinant protein binds 4 +/- 1 mol of cadmium/mol of protein and has a high tendency to form stable oligomeric structures. The structure of GSTdMT and dMT was investigated by synchrotron x-ray solution scattering and computational methods. X-ray scattering measurements indicated a strong tendency for GSTdMT to form dimers and trimers in solution and yielded structural models that were compatible with a stable dimeric form in which dMT had an extended conformation. Results of homology modeling and ab initio solution scattering approaches produced an elongated dMT structure with a long central hinge region. The predicted model and those obtained from x-ray scattering are in agreement and suggest that dMT may be involved in functions other than metal detoxification.

Birren, B. W. and H. R. Herschman (1986). "Regulation of the rat metallothionein-I gene by sodium butyrate." Nucleic Acids Res 14(2): 853-867.

Sodium butyrate selectively induces accumulation of metallothionein-I (MT-I) RNA in H4IIE rat hepatoma cells. The induction is rapid; significant elevation in cytoplasmic MT-I RNA can be observed within three hours after exposure to 5 mM butyrate. Maximal levels of MT-I RNA are obtained after eight hours. Butyrate stimulates MT RNA accumulation in the absence of de novo protein synthesis, indicating that MT induction by butyrate is not a distal step in a cascade of gene activation events. Butyrate blocks the induction of tyrosine amino transferase by dexamethasone. In contrast, butyrate and dexamethasone induced MT RNA elevations are additive. Butyrate induced MT-I RNA transcripts initiate at the correct start site. Measurements of the transcriptional activity of the MT-I gene indicate that butyrate stimulates MT-I transcription. The rapid, direct nature of the induction of MT-I by butyrate, combined with the extensive characterization of the metallothionein gene, provide an excellent system in which to study the effects of butyrate on a small, well-defined, responsive region of chromatin.

Blalock, T. L., et al. (1988). "Metallothionein gene expression in rats: tissue-specific regulation by dietary copper and zinc." J Nutr 118(2): 222-228.

Regulation of metallothionein gene expression by dietary zinc and copper was examined in rat liver, kidney, intestine and brain using a 3 X 3 factorial design. Purified diets containing 5, 30 and 180 mg Zn/kg and 1, 6 and 36 mg Cu/kg were fed for 2 wk. Serum concentrations of copper and zinc were lower at the lowest intakes of either metal than at normal or supplemental levels. Kidney metallothionein levels were proportional to dietary zinc, being 50% less in the 5 mg Zn/kg group than in those fed the highest zinc intake. Metallothionein mRNA was measured by dot blot hybridization to a 32P-labeled oligonucleotide DNA probe representing the terminal 5' sequence of the metallothionein gene. In kidney the number of metallothionein mRNA molecules per cell increased four- to five-fold (from 4 to 29 molecules per cell) with increasing dietary zinc. A less pronounced effect on metallothionein mRNA was observed in response to dietary copper. At the lowest copper intake level and highest intake of zinc intestinal metallothionein mRNA was sevenfold greater than in any other group. Liver and brain did not respond appreciably to the dietary levels of copper and zinc that were fed. Chromatography showed that copper and zinc content of renal metallothionein was directly related to the dietary levels fed. In kidney, both metallothionein-1 and -2 genes were expressed.

Boldrin, F., et al. (2008). "MTT2, a copper-inducible metallothionein gene from Tetrahymena thermophila." Comp Biochem Physiol C Toxicol Pharmacol 147(2): 232-240.

Metallothioneins (MTs) are ubiquitous, cysteine-rich, metal-binding proteins whose transcriptional activation is induced by a variety of stimuli, in particular heavy metals such as cadmium, copper and zinc. Here we describe the sequence and organization of a novel copper-inducible metallothionein gene (MTT2) from Tetrahymena thermophila. Based on its deduced sequence, the gene encodes a protein 108 amino acids, containing 29 cysteine residues (30%) arranged in motifs characteristic of vertebrate and invertebrate MTs. We demonstrate that the 5'-region of the MTT2 gene can act as an efficient promoter to drive the expression of heterologous genes in the Tetrahymena system. In the latter case, a gene for a candidate vaccine antigen against Ichthyophthirius multifiliis, a ubiquitous parasite of freshwater fish, was expressed at high levels in transformed T. thermophila cell lines. Moreover, the protein was properly folded and targeted to the plasma membrane in its correct three-dimensional conformation. This new copper-inducible MT promoter may be an attractive alternative to the cadmium-inducible MTT1 promoter for driving ectopic gene expression in Tetrahymena and could have a great impact on biotechnological perspectives.

Boldrin, F., et al. (2006). "Metallothionein gene from Tetrahymena thermophila with a copper-inducible-repressible promoter." Eukaryot Cell 5(2): 422-425.

We describe a novel metallothionein gene from Tetrahymena thermophila that has a strong copper-inducible promoter. This promoter can be turned on and off rapidly, making it a useful system for induction of ectopic gene expression in Tetrahymena and enhancing its applications in cell and molecular biology, as well as biotechnology.

Bonham, L., et al. (1991). "Inducible transformation of fibroblasts using a metallothionein-v-myc gene construct." Oncogene 6(6): 1073-1077.

An inducible oncogene construct has been engineered by coupling the MC29 v-myc oncogene to the sheep metallothionein promoter. Transfection of this plasmid, which also contains the neomycin resistance gene, into Rat-1 cells, has resulted in the isolation of independent clones resistant to G418. Certain of these clones were found to exhibit inducible transformation in response to ZnSO4. Transformation was graded with increasing ZnSO4 levels and was reversible when ZnSO4 was removed from the media. By analyzing v-myc mRNA levels, the inducible alterations in cellular morphology and growth were found to be associated with increased v-myc expression. The metallothionein promoter exhibited negligible constitutive expression of v-myc and none of the clones isolated exhibited spontaneous transformation. Our results show that the use of a metallothionein promoter v-myc construct facilitates the study of inducible fibroblast transformation.

Bonneton, F. and M. Wegnez (1995). "Developmental variability of metallothionein Mtn gene expression in the species of the Drosophila melanogaster subgroup." Dev Genet 16(3): 253-263.

Developmental expression of the Drosophila melanogaster metallothionein Mtn gene has been analysed. Transcripts of this gene accumulate during the vitellogenic phase of oogenesis in a ring of follicular cells at the oocyte-nurse cell margin and in the follicular cells surrounding the oocyte. There is also strong expression of the Mtn gene during the second half of embryogenesis in hemocytes, the endoderm midgut, and Malpighian tubules. A banded expression pattern is observed transiently in the midgut at stage 13. The two Mtn alleles, Mtn and Mtn, show quantitative differences in their expression patterns. Copper intoxication of flies does not induce ectopic expression of the Mtn gene, but rather leads to over-expression of the gene in the structures where it is normally transcribed. Mtn transcription is not altered in homozygous mutants of four genes (lab, wg, dpp, bap) known to be involved in midgut morphogenesis. Expression of Mtn has been also studied in six other species of the melanogaster subgroup. This analysis demonstrates that regulation of Mtn gene transcription has changed during evolution of the Drosophila lineage. For example, Mtn is expressed specifically in the Malpighian tubules of D. melanogaster, while in D. mauritiana and D. sechellia the amnioserosa is a specific location of expression. Nonetheless, expression of Mtn in the midgut is common to the seven species, suggesting a basic role for the MTN protein during embryogenesis in this organ, possibly in the release of metallic ions from vitellogenins. In contrast, two genes also expressed in the embryonic midgut, lab and dFRA, display identical patterns in all species of the melanogaster subgroup. The diversity of Mtn patterns in closely related Drosophila species exemplifies the rapid evolution of a gene regulatory system.

Bourdineaud, J. P., et al. (2006). "Challenging the model for induction of metallothionein gene expression." Biochimie 88(11): 1787-1792.

Metallothioneins (MTs) are low-molecular-weight, cysteine-rich metal-binding proteins found in a wide variety of organisms including bacteria, fungi and all eukaryotic plant and animal species. MTs bind essential and non-essential heavy metals. In mammalian cells MT genes are highly inducible by many heavy metals including Zn, Cd, Hg, and Cu. Aquatic systems are contaminated by different pollutants, including metals, as a result of man's activities. Bivalve molluscs are known to accumulate high concentrations of heavy metals in their tissue and are widely used as bioindicators for pollution in marine

and freshwater environments, with MTs frequently used as a valuable marker of metal contamination. We here describe the MT isoform gene expression patterns of marine and freshwater molluscs and fish species after Cd or Zn contamination. Contamination was carried out at a river site polluted by a zinc ore extraction plant or in the laboratory at low, environmentally relevant metal concentrations. A comparison for each species based on the accumulated MT protein levels often shows discrepancies between gene expression and protein level. In addition, several differences observed in the pattern of MT gene expression between mollusc and mammalian species enable us to discuss and challenge a model for the induction of MT gene expression.

Bourdineaud, J. P., et al. (2012). "Effects of methylmercury contained in a diet mimicking the Wayana Amerindians contamination through fish consumption: mercury accumulation, metallothionein induction, gene expression variations, and role of the chemokine CCL2." Int J Mol Sci 13(6): 7710-7738.

Methylmercury (MeHg) is a potent neurotoxin, and human beings are mainly exposed to this pollutant through fish consumption. We addressed the question of whether a diet mimicking the fish consumption of Wayanas Amerindians from French Guiana could result in observable adverse effects in mice. Wayanas adult men are subjected to a mean mercurial dose of 7 g Hg/week/kg of body weight. We decided to supplement a vegetarian-based mice diet with 0.1% of lyophilized Hoplias aimara fish, which Wayanas are fond of and equivalent to the same dose as that afflicting the Wayanas Amerindians. Total mercury contents were 1.4 +/- 0.2 and 5.4 +/- 0.5 ng Hg/g of food pellets for the control and aimara diets, respectively. After 14 months of exposure, the body parts and tissues displaying the highest mercury concentration on a dry weight (dw) basis were hair (733 ng/g) and kidney (511 ng/g), followed by the liver (77 ng/g). Surprisingly, despite the fact that MeHg is a neurotoxic compound, the brain accumulated low levels of mercury (35 ng/g in the cortex). The metallothionein (MT) protein concentration only increased in those tissues (kidney, muscles) in which MeHg demethylation had occurred. This can be taken as a molecular sign of divalent mercurial contamination since only Hg(2+) has been reported yet to induce MT accumulation in contaminated tissues. The suppression of the synthesis of the chemokine CCL2 in the corresponding knockout (KO) mice resulted in important changes in gene expression patterns in the liver and brain. After three months of exposure to an aimara-containing diet, eight of 10 genes selected (Sdhb, Cytb, Cox1, Sod1, Sod2, Mt2, Mdr1a and Bax) were repressed in wild-type mice liver whereas none presented a differential expression in KO Ccl2(-/-) mice. In the wild-type mice brain, six of 12 genes selected (Cytb, Cox1, Sod1, Sod2, Mdr1a and Bax) presented a stimulated expression, whereas all remained at the basal level of expression in KO Ccl2(-/-) mice. In the liver of aimara-fed mice, histological alterations were observed for an accumulated mercury concentration as low as 32 ng/g, dw, and metal deposits were observed within the cytoplasm of hepatic cells.

Brambila, E., et al. (2002). "Effect of mercury vapor exposure on metallothionein and glutathione s-transferase gene expression in the kidney of nonpregnant, pregnant, and neonatal rats." J Toxicol Environ Health A 65(17): 1273-1288.

Elemental mercury (Hg(0)) is a ubiquitous toxic pollutant. Exposure to Hg(0) vapor typically is by inhalation, and the kidney is the primary target organ. Glutathione (GSH) and metallothionein (MT) appear to mitigate mercury toxicity. However, little is known about GSH or MT regulation after Hg(0) vapor exposure, particularly during pregnancy, a time of high sensitivity to most metals. Thus, this study sought to determine renal mercury accumulation and MT- and GSH-related gene expression following Hg(0) vapor exposure in nonpregnant, pregnant, and neonatal rats exposed in utero. Groups (n = 5) of pregnant rats (Long-Evans) were exposed to Hg(0) vapor (4 mg/m(3)) or air (control) for 2 h/d from gestational day (GD) 6 to 15, and kidneys from dams and pups were removed at various times during and after the onset of exposure. For comparative purposes, nonpregnant female rats were exposed to Hg(0) for 10 d under the same conditions. Renal mercury, MT protein, and GST activity were assayed by standard analytical techniques. Western blot analysis was also performed using antibodies against MT and GST-pi. GSH-related gene expression was studied by cDNA microarray. Hg(0) vapor exposure produced renal accumulation of mercury in nonpregnant, pregnant, and neonatal rats. However, the transplacentally exposed neonates accumulated approximately 1000-fold less mercury than adults. Hg(0) vapor exposure produced a time-dependent increase in renal MT protein in nonpregnant and pregnant rats, but not in neonatal rats. Maximum MT increases were observed on d 10 (fivefold) in nonpregnant and GD 15 (threefold) in pregnant rats. Activation of the MT gene by Hg(0) was confirmed at the translational level by Western blot analysis and at the transcriptional level by Northern blot analysis. Microarray analysis revealed a significant upregulation in the renal expression of the GST-pi, GST-Ya, and microsomal GST and GST5-5 genes in nonpregnant and pregnant rats. Western blot and enzyme assay confirmed the upregulation of GST genes after Hg(0) exposure. Thus, in response to Hg(0) vapor exposure, the expression of the MT gene and various GST genes is activated in nonpregnant and pregnant rats.

Activation of these genes could be part of a defensive response directed at decreasing renal mercury toxicity, and may help divert the metal away from the fetus.

Brandle, J. E., et al. (1993). "Field performance and heavy metal concentrations of transgenic flue-cured tobacco expressing a mammalian metallothionein-beta-glucuronidase gene fusion." Genome 36(2): 255-260.

Cadmium (Cd) is a nonessential heavy metal that can cause acute and chronic illness in humans. Some plant species such as tobacco (Nicotiana tabacum L.) tend to accumulate high levels of Cd in leaf tissue, the consumed portion of the plant. Tissue-specific expression of mammalian metallothionein has been suggested as a means of partitioning Cd in nonconsumed portions of transgenic plants. The purpose of the experiment reported here was to evaluate Cd concentration and agronomic performance of four field-grown transgenic tobacco lines harbouring a metallothionein-beta-glucuronidase (MG) gene fusion driven by the constitutive 35S promoter of cauliflower mosaic virus. The trial was grown in a region of Canada known to have high background levels of Cd. The agronomic evaluation showed that some of the transgenic lines were equal to, while others performed more poorly than, the untransformed control for yield, days to flower, and leaf number. Gene expression measured by beta-glucuronidase activity showed that all of the transgenic lines expressed the MG gene in the upper portion of the plant. One line did not express the MG gene in the roots. Cd levels in the leaf tissue of transformed lines were not significantly different from the untransformed control.

Brazao-Silva, M. T., et al. (2015). "Metallothionein gene expression is altered in oral cancer and may predict metastasis and patient outcomes." Histopathology 67(3): 358-367.

AIMS: Metallothioneins (MTs) are proteins associated with the carcinogenesis and prognosis of various tumours. Previous studies have shown their potential as biomarkers in oral squamous cell carcinoma (OSCC). Aiming to understand more clearly the function of MTs in OSCC we evaluated, for the first time, the gene expression profile of MTs in this neoplasm. MATERIALS AND RESULTS: Tissue samples from 35 cases of tongue and/or floor of mouth OSCC, paired with their corresponding non-neoplastic oral mucosa (NNOM), were retrieved (2007-09). All tissues were analysed for the following genes using TaqMan((R)) reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays: MT1A, MT1B, MT1E, MT1F, MT1G, MT1H, MT1X, MT2A, MT3 and MT4. The expression of MT1B and MT1H was seldom detected in both OSCC and NNOM. A significant loss of MT1A, MT1X, MT3 and MT4 expression and gain of MT1F expression was observed in OSCC, compared to NNOM. Cases with MT1G down-regulation exhibited the worst prognoses. The up-regulation of MT1X was restricted to non-metastatic cases, whereas up-regulation of MT3 was related to cases with lymph node metastasis. CONCLUSIONS: Metallothionein mRNA expression is altered significantly in oral squamous cell carcinomas. The expression of MT1G, MT1X and MT3 may aid in the prognostic discrimination of OSCC cases.

Brkljacic, J. M., et al. (2004). "Expression analysis of buckwheat (Fagopyrum esculentum Moench) metallothionein-like gene (MT3) under different stress and physiological conditions." J Plant Physiol 161(6): 741-746.

The buckwheat metallothionein-like (MT3) gene expression was studied throughout seed and leaf development, as well as under the influence of different external stimuli. MT3 mRNAs were detected from the early stage of seed development to the end of maturation, reaching the highest level during the mid-maturation stage. High MT3 mRNA level was noticed for both green and senescent leaves. The influence of raising Cu ion concentrations on MT3 gene expression was studied only in leaves, while the effect of Zn ions was analyzed through seed development as well. It was found that Cu and Zn ions had stimulatory effects on expression in leaves. MT3 expression was significantly enhanced in the early stage of seed development in response to Zn ions, while after this stage, influence of Zn ions was not detected. After H2O2/NaCl treatment, MT3 mRNA level was decreased in green leaves, contrary to senescent leaves where expression levels remained unchanged. H2O2 treatment caused the increase of MT3 mRNA levels in the mid-maturation stage of seed development. NaCl had no effect on expression levels in seeds. According to obtained results, proposed functions in different plant organs regarding oxidative stress and metal homeostasis are discussed.

Brulle, F., et al. (2007). "The strong induction of metallothionein gene following cadmium exposure transiently affects the expression of many genes in Eisenia fetida: a trade-off mechanism?" Comp Biochem Physiol C Toxicol Pharmacol 144(4): 334-341.

Metal pollution causes disturbances at various levels of biological organization in most species. Important physiological functions could be affected in the exposed individuals and among the main physiological functions, immunity may provide one (or more) effector(s) whose expression can be directly affected by a metal exposure in various macroinvertebrates. Protein expressions were studied in order to test them as molecular biomarkers of metal exposure in Eisenia fetida. Selected effectors were

calmodulin, heat shock proteins, superoxide dismutase, catalase, metallothionein, beta-adrenergic receptor kinase, pyruvate carboxylase, transcriptionally controlled tumor protein, protein kinase C, ubiquitin and cyclophilin-A. The level of expression of each gene was analysed in whole organism following exposures to cadmium in soil using real-time PCR. Metallothionein, transcriptionally controlled tumor protein and cyclophilin-A expression were also measured following copper exposures in soil because these genes seemed to be sensitive to copper. This work enabled to distinguish metallothionein and cyclophilin-A among the 15 selected effectors. A strong decrease of the number of transcripts was also detected for most effectors soon after the exposure to cadmium suggesting that a trade-off mechanism occurs.

Brzezinski, R., et al. (1987). "Cloning and characterization of the metallothionein-I gene from mouse LMTK cells." Cytobios 52(208): 33-38.

A clone of about 14 kb containing the metallothionein MT-I gene and three repetitive sequences, was isolated from a genomic library of mouse LMTK DNA. The MT-I gene was functional. Transfected cells became cadmium resistant. Two of the three repetitive sequences were moderately repetitive while the other was closely related to the R family.

Buchanan-Wollaston, V. (1994). "Isolation of cDNA clones for genes that are expressed during leaf senescence in Brassica napus. Identification of a gene encoding a senescence-specific metallothionein-like protein." Plant Physiol 105(3): 839-846.

cDNA clones representing genes that are expressed during leaf senescence in Brassica napus were identified by differential screening of a cDNA library made from RNA isolated from leaves at different stages of senescence. The expression of these genes at different stages of leaf development was examined by northern blot analysis, and several different patterns of expression were observed. One of the clones, LSC54, represented a gene that is expressed at high levels during leaf senescence. Analysis of this gene indicated strong expression in flowers as well as in senescing leaves. DNA sequence analysis of the LSC54 cDNA indicated a similarity between the deduced amino acid sequence and several metallothionein-like proteins previously identified in plants.

Buchman, C., et al. (1989). "The CUP2 gene product, regulator of yeast metallothionein expression, is a copper-activated DNA-binding protein." Mol Cell Biol 9(9): 4091-4095.

CUP2 is a regulatory gene controlling expression of CUP1, which encodes the Cu-binding yeast metallothionein. CUP2, which is identical to the ACE1 gene, encodes a Cu-regulated DNA-binding protein. The CUP2 protein contains a cysteine-rich DNA-binding domain dependent on Cu+ and Ag+ ions which bind the cysteine residues and direct the refolding of the metal-free apoprotein. CUP2 mutant alleles from Cu-sensitive yeast strains have point mutations affecting the DNA-binding activity. These results establish CUP2 as the primary sensor of intracellular Cu+ in the yeast Saccharomyces cerevisiae, functioning as a Cu+-regulated transcriptional activator.

Butt, A., et al. (1998). "Differential expression of a senescence-enhanced metallothionein gene in Arabidopsis in response to isolates of Peronospora parasitica and Pseudomonas syringae." Plant J 16(2): 209-221.

The metallothionein gene, LSC54, shows increased expression during leaf senescence in Brassica napus and Arabidopsis thaliana. A number of abiotic and biotic stresses have been shown to induce senescence-like symptoms in plants and, to investigate this further, the promoter of the LSC54 gene was cloned and fused to the GUS gene and transformed into Arabidopsis. The promoter was highly induced during leaf senescence and also in response to wounding; histochemical analysis indicated that this induction was localised to a few cells close to the wound site. The transgenic Arabidopsis tissue was infected with compatible and incompatible isolates of both the fungal biotroph, Peronospora parasitica and the bacterial necrotroph, Pseudomonas syringae. Incompatible isolates induced rapid cell death (the hypersensitive response) at the site of infection and, with both pathogens, early, localised expression of the GUS gene was observed. In contrast, relatively slow induction of the GUS gene was seen in the compatible interaction and this was correlated with the appearance of senescence-like symptoms in the biotrophic interaction and cell death by necrosis that occurred in response to the necrotrophic pathogen. These results suggest that there are common steps in the signalling pathways that lead to cell death in the hypersensitive response, pathogen induced necrosis and senescence.

Butt, T. R., et al. (1984). "Cloning and expression of a yeast copper metallothionein gene." Gene 27(1): 23-33.

The induction of a copper-binding metallothionein (Cu-MT) was studied in yeast, Saccharomyces cerevisiae, and a relationship between copper resistance and intracellular levels of Cu-MT in these eukaryotes was established. Poly(A)-containing RNA from a copper-resistant (Cur) yeast strain, which synthesized abundant quantities of Cu-MT and in which Cu-MT gene transcription was enhanced 50-fold upon exposure to CuSO4, was used to screen yeast genomic DNA clones. Restriction analysis revealed common XbaI and KpnI sites in five genomic clones isolated. The transcription of these clones was regulated by copper. Transformation of a copper-

sensitive (Cus) yeast strain by one of these clones confers copper resistance in yeast. The results suggest that the expression of the Cu-MT gene is, in part, responsible for mediating copper resistance in yeast.

Butt, T. R., et al. (1984). "Copper metallothionein of yeast, structure of the gene, and regulation of expression." Proc Natl Acad Sci U S A 81(11): 3332-3336.

Addition of copper to yeast cells leads to the induction of a low molecular weight, cysteine-rich protein that binds copper. This protein, termed copper chelatin or thionein, is related to the metallothionein family of proteins that are induced in response to cadmium and zinc in vertebrate cells. We have determined the structure of the yeast copper-binding protein by DNA sequence analysis of the gene. Although the 6573-dalton yeast protein is substantially divergent from vertebrate metallothioneins, the arrangement of 12 cysteine residues, which is a hallmark of metal-binding proteins, is partially conserved. We analyzed the regulatory DNA sequence of the gene by fusing it with the Escherichia coli galactokinase gene and assaying the levels of enzyme activity in yeast in response to copper. The transcriptional activation has a specific requirement for copper. Zinc, cadmium, and gold were unable to regulate the galactokinase activity. The yeast copper metallothionein regulatory sequences represent a previously unreported class of yeast promoter that is regulated by copper.

Butt, T. R., et al. (1986). "Regulation of metallothionein gene expression in mammalian cells by gold compounds." Mol Pharmacol 29(2): 204-210.

Metallothioneins are a class of low molecular weight, cysteine-rich proteins. Metallothioneins bind heavy metals and are thought to play a role in metal metabolism. Auranofin, an antiarthritic gold compound, is a potent inducer of metallothionein in Chinese hamster ovary cells. The induction of metallothionein by auranofin was mediated by active transcription of the gene and new mRNA was accumulated within 30 min after the exposure of Chinese hamster ovary cells to the drug. The extent of metallothionein induction was related to the concentration of the compound and was affected by the nature of the ligand attached to the gold molecule. A subline of these Chinese hamster ovary cells was established by growing them in the presence of normally cytotoxic concentrations of auranofin. In this auranofin-resistant cell line, the metallothionein genes were actively transcribed in the presence of auranofin, suggesting a relationship between cytotoxic action of auranofin and metallothionein gene transcription. Regulation of metallothionein gene transcription may play an important role in the molecular mechanism(s) of action auranofin and resistance to it.

Carri, M. T., et al. (1991). "Evidence for co-regulation of Cu,Zn superoxide dismutase and metallothionein gene expression in yeast through transcriptional control by copper via the ACE 1 factor." FEBS Lett 278(2): 263-266.

Saccharomyces cerevisiae mutant strain DTY26, lacking ACE1, the protein mediator for the induction of metallothionein gene expression, is unable to increase Cu,Zn superoxide dismutase mRNA in response to copper. In the wild-type strain DTY22 transcription of both Cu,Zn superoxide dismutase and metallothionein genes is induced by copper and silver, as expected on the basis of previous results indicating that ACE1 binds only Ag(I) besides Cu(I). We conclude that at the transcriptional level Cu,ZnSOD is co-regulated with metallothionein. Furthermore, structural similarities between the two promoters were found, which could explain the co-regulation effect and the quantitative differences in the response of the two genes to copper.

Carter, A. D., et al. (1984). "Duplicated heavy metal control sequences of the mouse metallothionein-I gene." Proc Natl Acad Sci U S A 81(23): 7392-7396.

We present evidence that two distinct regions of the DNA upstream from the mouse metallothionein-I gene contain metal-responsive regulatory sites. This result was obtained by analyzing a systematic series of deletion, insertion, duplication, and clustered point mutations introduced into cultured cells on a simian virus 40 plasmid vector. The two upstream regions contain a duplicated evolutionarily conserved DNA sequence. While either upstream region is sufficient to confer heavy metal responsiveness, both are required to give maximal levels of induced transcription.

Castiglione, S., et al. (2007). "High zinc concentrations reduce rooting capacity and alter metallothionein gene expression in white poplar (Populus alba L. cv. Villafranca)." Chemosphere 67(6): 1117-1126.

Poplar is a good candidate for phytoremediation purposes because of its rapid growth, extensive root system, and ease of propagation and transformation; however its tolerance to heavy metals has not been fully investigated yet. In the present work, an in vitro model system with shoot cultures was used to investigate the tolerance to high concentrations of zinc (Zn) of a commercial clone (Villafranca) of Populus alba. Based on chlorophyll content (leaf chlorosis) and the rate of adventitious root formation from shoot cuttings as parameters of damage, 0.5-4mM zinc concentrations were all toxic albeit to different extents. Northern blot and reverse transcriptase (RT)-PCR analyses were used to examine the expression profiles of types 1, 2 and 3 PaMT genes in stems, leaves and roots of plants exposed to Zn treatments. In leaves, MT1 and MT3 mRNA levels were enhanced by Zn, while MT2 transcripts were not affected. The

PaMT expression profiles were differentially affected by Zn in an organ-specific manner, and the relationship with Zn concentration and exposure time was rarely linear. The developmental and molecular data reveal that the in vitro model is a sensitive and reliable system to study heavy metal stress responses.

Ceratto, N., et al. (2002). "Cloning and sequencing of a novel metallothionein gene in Mytilus galloprovincialis Lam." Comp Biochem Physiol C Toxicol Pharmacol 131(3): 217-222.

Metallothionein (MT) is a ubiquitous, metal-inducible protein with an important role in the homeostasis and in the detoxification of heavy metals. This work reports the cloning and sequencing of a MT gene encoding a MT isoform (MT20-IIIa) in the mussel Mytilus galloprovincialis Lam, a lamellibranch mollusc known to accumulate and to detoxify large amounts of metal. The MT gene, lacking the 5' promoter region, is 1865 bp long and has a tripartite structure consisting of three exons and two introns. The putative open reading frame (ORF) encodes a polypeptide of 72 amino acids, which corresponds to the MT-I class, type 2 family (http://www.unizh.ch/~mtpage/classif.html). The structure of the gene and the putative MT20-III protein have been compared with those of other species. The putative biological significance of the differences at the amino acid level among the different MTs is discussed.

Chan, K. M., et al. (2006). "Metallothionein gene expression in zebrafish embryo-larvae and ZFL cell-line exposed to heavy metal ions." Mar Environ Res 62 Suppl: S83-87.

The aim of this study is to investigate the induction of zebrafish metallothionein (zMT) gene expression following the administration of different metal ions using in vivo and in vitro models. The zebrafish embryo-larvae were used for the in vivo study, and MT gene expression was studied during the development from fertilization (8hpf) to embryo-larval stage using real-time PCR. The LC50 values and zMT mRNA levels were also measured in embryo-larvae exposed to various metal ions. The general trend of 24 h LC50 values as determined is Cu2+ < Hg2+ < Cd2+ << Zn2+. However, Hg2+ was found to be the most potent metal inducer with the highest level of zMT mRNA induction (40-50 folds) in 8hpf embryo-larvae, followed by Cd2+ (approximately 20 folds); Cu2+ and Zn2+ only gave approximately 5 fold of induction. In the in vitro study of ZFL cell-line, Cd2+ is the most potent inducer of zMT mRNA (up to 250 folds), Cu2+ and Zn2+ gave similar potency of approximately 50-100 folds, and Hg2+ gave approximately 40-50 folds of zMT mRNA levels over the control group.

Chan, M. K., et al. (2002). "Induction of a putative metallothionein gene in the blood cockle, Anadara granosa, exposed to cadmium." Comp Biochem Physiol C Toxicol Pharmacol 131(2): 123-132.

The relationship between a putative metallothionein gene (MT) and exposure to cadmium (Cd) in blood cockles (Anadara granosa) is reported. In a 96-h dose-response experiment, mortality of cockles was found to proportionately increase in the range of 0.2-5.0 mg/l Cd with a calculated LC(50) of 2.94 mg/l. Exposure to 0.25 mg/l Cd for 16 days caused significant increases ($P<0.05$) in Cd concentrations in whole tissues, gills and hepatopancreas, and the accumulation of Cd in these tissues increased with the duration of exposure. Two cDNA libraries constructed using the hepatopancreas from control and Cd-treated cockles gave titres of 5.62 x 10(5) and 1.94 x 10(5) pfu/microg vector, respectively. A putative MT gene, AnaMT, of 510 nucleotides in length, was isolated from the treated cDNA library using a heterologous probe MT20 from the blue mussel, Mytilus edulis. Northern analyses using AnaMT as a probe indicated low expression of the MT mRNA in control animals. In cockles treated with 0.25 mg/l Cd for 4 days, MT mRNA level increased to approximately 168%, but declined to 108% at day 8. After 12 and 16 days of Cd treatment, expression of the MT gene was 138% and 187%, respectively, compared to the controls. These observations suggest that induction of the MT gene by a sublethal dose of Cd is rapid, occurring within 4 days of treatment.

Chan, P. C., et al. (2004). "Common carp metallothionein-1 gene: cDNA cloning, gene structure and expression studies." Biochim Biophys Acta 1676(2): 162-171.

Metallothionein-1 (MT-1) cDNA clones were isolated from a common carp (Cyprinus carpio) uninduced hepatopancreas cDNA library. Northern blot assay using the common carp (cc) MT-1 cDNA as a probe showed high fold induction of ccMT mRNA levels in the intestine and kidney following exposure to Cd2+ and Zn2+. Using polymerase chain reaction (PCR), primers designed from the cDNA sequences allowed the isolation of ccMT-1 gene fragments including the 5'-flanking region. The 600 bp 5'-flanking region of ccMT-1 gene carries four putative metal regulatory regions, one AP1, two SP1, one c-Jun site, and a TATA box. The 5'-flanking region of the ccMT-1 gene obtained was a functional promoter responding to the administration of various metal ions as well as hydrogen peroxide (H2O2) and lipopolysaccharide (LPS). When tested in primary cultures of cc hepatocytes, Zn2+ had the highest fold (20 times) induction of the 600 bp cloned ccMT-1 gene promoter, followed by Cu2+, Hg2+, Ni2+ and Pb2+ (4-5-fold inductions); H2O2 and LPS had a 6-7-fold induction. In conclusion, the ccMT-1 is a constitutively expressed MT and its gene promoter is inducible by various metal ions and chemical agents.

Chan, W. W. and K. M. Chan (2008). "Cloning and

characterization of a tilapia (Oreochromis aureus) metallothionein gene promoter in Hepa-T1 cells following the administration of various heavy metal ions." Aquat Toxicol 86(1): 59-75.

Metallothioneins (MTs) are highly conserved intracellular metal-binding proteins that contribute to the homeostasis of essential metals and the detoxification of non-essential heavy metals. MT gene expression is induced by various heavy metal ions, and Zn(2+) is able to bind and activate a transcription factor associated with the MT gene that is known as the metal responsive element (MRE) binding transcription factor-1 (MTF-1). Heavy metals other than Zn(2+), such as Cd(2+) and Cu(2+), fail to activate the binding of MTF-1 to MREs despite their ability to induce the transcription of the MT gene. To study how different metal ions regulate MT gene expression, a tilapia (ti)-MT gene promoter was cloned and its responses to activation by various metal ions measured using a Hepa T1 cell culture model. The tiMT gene promoter contains six functional MREs within 2118bp 5' of the translational start site. A transient gene expression study showed the tiMT gene promoter fragment to be responsive to Cd(2+), Cu(2+), Hg(2+), Pb(2+), and Zn(2+). Deletions from the 5' end and the site-directed mutagenesis of individual MREs in the tiMT gene promoter confirmed that both proximal and distal clusters of MREs were required for the maximal metal induction of the tiMT gene. The distal cluster of MREs greatly enhanced the induction of tiMT gene expression by several of the heavy metal ions, and especially the non-Zn(2+) ions. Individual MREs showed a different responsiveness to metal ions, with MREe being the most potent, MREb being responsive to Zn(2+) but not to other metal ions, and MREa being mainly for the basal expression of the tiMT gene. Electrophoretic mobility shift assay (EMSA) identified a transcription factor that was able to bind most of the MREs, with the exception of MREd, but the binding was only activated by the in vivo administration of Zn(2+), not the administration of Cd(2+) or Cu(2+). In conclusion, the results of this study on a Hepa T1 cell model suggest that the mechanism of MT gene activation by non-Zn(2+) metal ions is different from that of activation by Zn(2+), and that different MREs may be involved in the activation of the tiMT gene by different metal ions without enhancing the binding of MTF-1 to MREs.

Chang, C. Y., et al. (2019). "Identification of sugar response complex in the metallothionein OsMT2b gene promoter for enhancement of foreign protein production in transgenic rice." Plant Cell Rep 38(8): 899-914.

KEY MESSAGE: A 146-bp sugar response complex MTSRC is identified in the promoter of rice metallothionein OsMT2b gene conferring high-level expression of luciferase reporter gene and bioactive recombinant haFGF in transgenic rice. A rice subfamily type 2 plant metallothionein (pMT) gene, OsMT2b, encoding a reactive oxygen species (ROS) scavenger protein, has been previously shown to exhibit the most abundant gene expression in young rice seedling. Expression of OsMT2b was found to be regulated negatively by ethylene and hydrogen peroxide in rice stem node under flooding stress, but little is known about its response to sugar depletion. In this study, transient expression assay and transgenic approach were employed to characterize the regulation of the OsMT2b gene expression in rice. We found that the expression of OsMT2b gene is induced by sugar starvation in both rice suspension cells and germinated embryos. Deletion analysis and functional assay of the OsMT2b promoter revealed that the 5'-flanking region of the OsMT2b between nucleotides - 351 and - 121, which contains the sugar response complex (- 266 to - 121, designated MTSRC) is responsible for high-level promoter activity under sugar starvation. It was also found that MTSRC significantly enhances the Act1 promoter activity in transgenic rice cells and seedlings. The modified Act1 promoter, Act1-MTSRC, was used to produce the recombinant human acidic fibroblast growth factor (haFGF) in rice cells. Our result shows that the bioactive recombinant haFGF is stably produced in transformed rice cell culture and yields are up to 2% of total medium proteins. Our studies reveal that MTSRC serves as a strong transcriptional activator and the Act1-MTSRC promoter can be applicable in establishing an efficient expression system for the high-level production of foreign proteins in transgenic rice cells and seedlings.

Chang, T., et al. (2004). "A metallothionein-like gene htMT2 strongly expressed in internodes and nodes of Helianthus tuberosus and effects of metal ion treatment on its expression." Planta 218(3): 449-455.

A cDNA sequence encoding a type-2 metallothionein (MT)-like protein, designated htMT2, was isolated from a Helianthus tuberosus L. tuber cDNA library. The isolated cDNA is 509 bp, coding a 7.8-kDa polypeptide. Two partial genomic fragments covering the open reading frame of htMT2 were cloned by PCR. The fragments htMTG-1 (986 bp) and htMTG-2 (982 bp) contain three exons and two introns. The N- and C-terminal domains of the predicted polypeptide have eight and seven cysteine residues, separated by a central cysteine-free spacer. Sequence alignment revealed that the predicted protein was homologous to type-2 MTs of plants. Southern blot analysis indicated that htMT2 is encoded by a small multi-gene family in the H. tuberosus genome. Northern blot analysis showed that htMT2 transcripts were predominantly expressed in internodes and nodes, but were low in leaves, leafstalks, tubers and young

roots, and none was detected in roots. Treatment with Cu(2+) reduced the expression of htMT2 in internodes, nodes, leaves and leafstalks. In addition, the expression levels in internodes and nodes share an inverse relationship with the concentrations of Cu(2+). In internodes and nodes, treatment with Zn(2+) at 10 and 100 microM reduced the expression levels of htMT2, and 1000 microM Zn(2+)reduced it to the lowest level, but 500 microM Zn(2+) had little effect. The expressions of htMT2 in different tissues were not appreciably affected by heat shock. It is suggested that HtMT2 might be involved in the transport or availability of Cu(2+) and Zn(2+) to some apo-metal enzymes or apo-metal proteins.

Chang, X. L., et al. (2006). "[Application of metallothionein gene isoforms expression as biomarkers in cadmium exposure]." Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 24(1): 12-15.

OBJECTIVE: To investigate the feasibility of metallothionein (MT) gene expression level in human peripheral blood lymphocytes (HPBLs) as a biomarker in cadmium exposure. METHODS: The MT gene expression level in HPBLs of workers exposed to cadmium was examined using RT-PCR technique, and the exposure assessment and effect assessment were conducted in exposed workers. RESULTS: The basal MT-1A, IE, IF, IX and MT-2A expression level in workers exposed to cadmium were significantly higher than those in the control group ($P < 0.05$). The basal MT-1A, IE, IF, IX and MT-2A expression level would be significantly increased with the increase of the blood cadmium (BCd) level ($P < 0.05$). There was a trend of increase for the mRNA expression of the basal MT-1A, 1E, IF, IX, MT-2A, especially for the mRNA expression of MT-1A and MT-2A ($P < 0.05$) with the increase of the level of the urine cadmium (UCd). There was a good dose-response relationship between basal MT-1A expression and UCd. The basal MT-1A, IE, IF, IX and MT-2A expression level were significantly correlated with BCd ($P < 0.05$) while the basal MT-1A, IF and MT-2A expression level were significantly correlated with UCd ($P < 0.05$). There were dose-effect relationships of BCd to the basal MT-1E, MT-1F, MT-1X and MT-2X expression level respectively and there were also dose-effect relationships of UCd, beta(2)-MG and the urine metallothionein to the basal MT-1A expression. CONCLUSION: The expression of the MT gene isoforms in HPBLs can serve as the biomarker for the cadmium exposure and MT-1A can also serve as the effective biomarkers for the cadmium-induced renal toxicity.

Chang, X. L., et al. (2005). "[Metallothionein isoforms gene expression induced by cadmium in human peripheral blood lymphocytes]." Wei Sheng Yan Jiu 34(2): 137-140.

OBJECTIVE: To explore gene expression of metallothionein 1 (MT-1) isoforms in human peripheral blood lymphocytes. METHODS: mRNA of expression representing the seven active subtypes of MT-1 gene was determined in cultured human peripheral blood lymphocytes (HPBLs) before and after exposure to cadmium by quantitative RT-PCR. RESULTS: Basal expression of MT-1X, MT-1A in HPBLs was similar to expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase. In contrast, the basal gene expression of MT-1H, MT-1F, MT-1E, MT-1G was a little transcript in HPBLs. No signal was detected for the MT-1B. There was a sex difference ($P < 0.05$) in basal gene expression of MT-1E. The level of gene expression of MT-1A, MT-1E, MT-1F, MT-1G, MT-1H and MT-1X was significantly increased ($P < 0.05$), but not the level of MT-1B after exposure to cadmium. CONCLUSION: It was suggested that gene expression of MT-1F, MT-1G, MT-1H and 1X in HPBLs could be used as potential exposure biomarker.

Chang, X. L., et al. (2006). "Metallothionein 1 isoform gene expression induced by cadmium in human peripheral blood lymphocytes." Biomed Environ Sci 19(2): 104-109.

OBJECTIVE: To study the gene expression of metallothionein 1 (MT-1) isoforms in human peripheral blood lymphocytes (HPBLs). METHODS: The expression of mRNA representing the seven active MT-1 genes was determined in HPBLs by quantitative RT-PCR before and after exposure to cadmium. RESULTS: Basal expressions of MT-1X, and MT-1A in HPBLs were similar to expression of housekeeping gene. In contrast, the basal gene expressions of MT-1H, 1F, 1E, and 1G were a little transcripts in human HPBLs. No signal was detected for MT-1B. There was a sex difference ($P < 0.05$). in basal gene expression of MT-1E. The levels of gene expression of MT-1A, 1E, 1F, 1G, 1H, and 1X increased, but the level of MT-1B did not increase after exposure to cadmium. CONCLUSIONS: Gene expressions of MT-1G, MT-1H, MT-1F, and MT-1X in HPBLs can be used as a potential biomarker of cadmium exposure.

Chang, Y., et al. (2011). "[Function comparison and evolution analysis of metallothionein gene MTT2 and MTT4 in Tetrahymena thermophila]." Dongwuxue Yanjiu 32(5): 476-484.

Tetrahymena has a high genetic polymorphism of metallothionein proteins. These protein isoforms can be divided into subfamilies 7a and 7b. We used real-time quantitative PCR to test the expression levels of Tetrahymena thermophila metallothionein genes MTT2 and MTT4 after exposure to different inducers, including Hg, Cu, Cd, Zn and H(2)O(2). Both genes were most efficiently induced by Cu and secondly by Hg. Their expression was slightly up-regulated after exposure to Cd and Zn, but down-regulated by

H(2)O(2). The expression pattern differed from those reported in the 7a subfamily, but was consistent with 7b subfamily members. However, the induced expression level of MTT4 was much higher than that of MTT2, which might be due to differences in their upstream regulatory elements (AP-1, MRE). The high similarities in gene structures and functions indicate that MTT2 and MTT4 were generated by recent gene duplication following the dosage balance model.

Charbonnel-Campaa, L., et al. (2000). "Isolation of a type 2 metallothionein-like gene preferentially expressed in the tapetum in Zea mays." Gene 254(1-2): 199-208.

A Zea mays cDNA, MZm3-4, was isolated by differential screening of a cDNA library obtained from meiotic stage anthers against a cDNA of 3-week-old seedlings. Northern blot analysis of RNA from different maize tissues and from male reproductive organs at various developmental stages demonstrated expression of a single transcript in anthers, from the pollen mother cell stage through the uninucleated microspore stage. In situ hybridization to anther sections resulted in a distinct signal only in the tapetum. The MZm3-4 cDNA is 743 nucleotides in length and has an open reading frame encoding a protein of 75 amino acids. Sequence comparisons with various databases revealed that MZm3-4 exhibits high similarities with type 2 plant metallothioneins at both the nucleotide and the amino-acid level. Primer extension analysis indicated that MZm3-4 cDNA is deleted of 13bp at the 5' end. Southern blot analysis showed that the MZm3-4 gene may be present in one or two copies in a Z. mays inbred line genome. This is the first report of the isolation of a type 2 metallothionein-like protein in maize. Moreover, the expression of this type 2 metallothionein-like gene is high in the male reproductive organs engaged in microsporogenesis.

Chatthai, M., et al. (2004). "Functional analysis of a Douglas-fir metallothionein-like gene promoter: transient assays in zygotic and somatic embryos and stable transformation in transgenic tobacco." Planta 220(1): 118-128.

Douglas-fir (Pseudotsuga menziesii [Mirb] Franco) metallothionein (PmMT) cDNA encodes a novel cysteine- and serine-rich MT, indicating a new subtype or prototype MT from which other plant MTs may have evolved. A genomic library of Douglas-fir was screened using MT cDNA probes, and genomic sequences that mediate tissue-specific, temporal as well as inducible expression of the embryo-specific MT-gene were analyzed. The promoter region of the PmMT genomic clone (gPmMT) contained a hexameric G-box, two putative ethylene-responsive elements and an inverted repeat of a motif similar to the core metal regulatory element. Interestingly, comparison of the upstream region of Douglas-fir gPm2S1 and gPmMTa genes revealed a conserved motif, CATTATTGA, not found in any known angiosperm gene promoter. Chimeric gene constructs containing a series of deletions in the gPmMTa promoter fused to the uidA reporter gene were assayed in Douglas-fir and transgenic tobacco (Nicotiana tabacum L.). Transient-expression assays in Douglas-fir megagametophyte and zygotic embryos indicated that the sequence -190 to +88 of gPmMTa was sufficient to drive the expression of the reporter gene and that the 225-bp fragment (-677 to -453) contained sequences necessary for high-level expression. In transgenic tobacco seedlings the beta-glucuronidase activity was localized in the vacuolar tissue and proliferating tissue of the auxiliary buds and stem elongation zone. The gPmMTa promoter was not active in the seeds of transgenic tobacco or in the roots of seedlings up to 3 weeks old. Detailed studies of transient expression and stable transformation provided important information on evolutionary conservation as well as novel features found in the conifer promoter. This is the first report of an MT-like gene promoter from conifers.

Chaturvedi, A. K., et al. (2012). "Cloning and transcript analysis of type 2 metallothionein gene (SbMT-2) from extreme halophyte Salicornia brachiata and its heterologous expression in E. coli." Gene 499(2): 280-287.

Salicornia brachiata is an extreme halophyte growing luxuriantly in the coastal marshes and frequently exposed to various abiotic stresses including heavy metals. A full length type 2 metallothionein (SbMT-2) gene was isolated using RACE and its copy number was confirmed by southern blot analysis. Transcript expression of SbMT-2 gene was analyzed by semi-quantitative Rt-PCR and real time quantitative (qRT) PCR. Expression of SbMT-2 gene was up-regulated concurrently with zinc, copper, salt, heat and drought stress, down regulated by cold stress while unaffected under cadmium stress. Heterologous expression of SbMT-2 gene enhances metal accumulation and tolerance in E. coli. Metal-binding characteristics of SbMT-2 protein show its possible role in homeostasis and/or detoxification of heavy metals. Significant tolerance was observed by E. coli cells expressing recombinant SbMT-2 for Zn(++), Cu(++) and Cd(++) compared to cells expressing GST only. Sequestration of zinc was 4-fold higher compared to copper and in contrast SbMT-2 inhibits the relative accumulation of cadmium by 1.23-fold compared to GST protein. Fusion protein SbMT-2 showed utmost affinity to zinc (approx. 2.5 fold to Cu(++) and Cd(++)) followed by copper and cadmium ions with same affinity. Halophyte S. brachiata has inherent resilience of varying abiotic tolerance therefore SbMT-2 gene

could be a potential candidate to be used for enhanced metal tolerance and heavy metal phytoremediation.

Chaudhry, R. and A. R. Shakoori (2010). "Isolation and characterization of a novel copper-inducible metallothionein gene of a ciliate, Tetrahymena tropicalis lahorensis." J Cell Biochem 110(3): 630-644.

The two isoforms of copper metallothionein (CuMT) gene of a copper resistant ciliate, Tetrahymena tropicalis lahorensis (Ttl), have been isolated and characterized. The molecular cloning and nucleotide sequencing of cDNAs coding for the two CuMT isoforms revealed that TtlCuMT1 gene has 300, while TtlCuMT2 has 327 nucleotides, both with ATG as the initiation codon and TGA as the translational termination codon. TAG codes for glutamine in TtlCuMT2 gene which is peculiar to Tetrahymena. The deduced or translated TtlCuMT1 and TtlCuMT2 peptide sequences contain 100 and 108 amino acid residues including 28 and 32 cysteine residues, respectively. The amino acid sequences of TtlCuMT1 and TtlCuMT2 have special features of two and three CXCXXCXCXXCXC intragenic tandem repeats with a conserved structural pattern of cysteine, respectively. The predicted tertiary structures of these two isoforms indicate two domains. Domain I and the initial part of domain II showed >98% homology with other Tetrahymena CuMT. On the basis of the differences in the domain II, the metallothionein subfamily 7b can be divided into two groups, one (TtlCuMT1) comprising >100 amino acids and the other (TtlCuMT2) comprising <100 amino acids. This is a novel finding of the present study as no such report on this type of classification exists at the moment. TtlCuMT1 has 95%, while TtlCuMT2 has 97% resemblance with the previously reported CuMT genes of Tetrahymena spp. SDS-PAGE analysis using fluorescent probe as well as coomassie brilliant blue staining also confirmed the presence of metallothionein.

Chen, H. I., et al. (2010). "The association of metallothionein-4 gene polymorphism and renal function in long-term lead-exposed workers." Biol Trace Elem Res 137(1): 55-62.

The goal of this study is to investigate if metallothionein (MT) gene polymorphism affects the susceptibility to lead as well as renal function parameters and blood pressures (BP) in workers exposed to lead for extended period of time. By means of real-time polymerase chain reaction, the MT4-216 A/G genotypes classified as rs396230 in the single nucleotide polymorphism database of the National Center for Biotechnology Information (database) were analyzed on 113 workers of a lead battery-recycling factory. Workers with G (mutant) allele were more susceptible to the toxic effects of lead on their systolic BP and serum renal function parameters. Their BP was 10 mmHg higher than those with wild-type (AA type) allele. Among subjects with the 3-genome, the GG mutant type subjects appear to be more susceptible to lead. Regression models of serum creatinine and BUN showed significant differences between the GG and GA types compared to AA type subjects. This cross-sectional study shows that workers with different MT-4 genotypes have different lead-induced adverse health effects. Those with the G allele have the greater susceptibility to lead so their exposure should be reduced.

Chen, J. H., et al. (2001). "Rottlerin stimulates metallothionein gene expression but inhibits metal transport in Chinese hamster ovary cells." Toxicol Appl Pharmacol 177(3): 256-263.

Metallothionein (MT) can be induced by various metals. We have shown previously that H7, a protein kinase C (PKC) inhibitor, inactivates metal-induced MT gene expression. To investigate whether a specific PKC isoform is involved in the induction process, inhibitors for various PKC isoforms were administered to cadmium-resistant Chinese hamster ovary (Cd(R)) cells. None of the inhibitors used can reduce metal-induced MT gene expression. However, a PKCdelta inhibitor, rottlerin, induced MT mRNA expression in Cd(R) cells in the presence or absence of Cd. Notably, the induction occurs through the activation of the MT transcriptional factor (MTF-1) and is not related to an increase of metal influx. Furthermore, metal accumulation is reduced in the presence of rottlerin. Pulse-labeling analysis indicated that MT protein synthesis increased in Cd(R) cells upon rottlerin treatment. These results suggest that rottlerin blocks metal transport but stimulates MT synthesis in Cd(R) cells. Since rottlerin is capable of reducing the cellular accumulation of Cd, it was expected that the cytotoxic effect of Cd would decrease in the presence of rottlerin. Treating the parental cell of Cd(R) with Cd and rottlerin together indeed showed a decline of cytotoxicity compared to cells treated with Cd alone. We further examined how MTF-1 was activated by rottlerin. Rottlerin-induced MTF-1 activity was not affected in Cd(R) cells by the addition of EDTA. It was, however, diminished by administering an intracellular Zn chelator, TPEN. The result implies a mobilization of intracellular Zn ions after rottlerin treatment in Cd(R) cells. To investigate whether the described results occur in all types of cells, another cell line (GH(3)) was used to study the effect of rottlerin on MT gene expression. The result revealed that rottlerin did not increase the amount of MT mRNA in GH(3) cells. This differential effect between cell lines may be useful for investigating the regulatory mechanism of MT gene expression.

Chen, W. M., et al. (1998). "Type 2 rice metallothionein-like gene has two introns." DNA Seq 8(4): 223-228.

A type 2 rice metallothionein-like gene was isolated from root by PCR and sequenced. The PCR fragment was designated as pcr1460, which overlaps with OsMT-2, a cDNA sequence previously characterized, with the presence of two additional segments, 583 and 613 bp in length. These segments are recognized as introns which divide the coding sequence into three exons, 65, 78 and 106 base pairs in length. The sequences flanking the introns conform with the GT/AG rule for splice junctions, and one exonic open reading frame can be identified in each of the introns. The observation that MT-like gene has two introns is the first of such a finding obtained from monocotyledonous plants.

Chen, W. Y., et al. (2004). "Expression of metallothionein gene during embryonic and early larval development in zebrafish." Aquat Toxicol 69(3): 215-227.

Metallothionein (Mt) has been considered as a molecular marker of metal pollution in aquatic ecosystems. Less is known about the expression of mt gene during embryogenesis. Here, we report the cloning, sequencing, and the expression pattern of mt gene during developmental stages in zebrafish. The zebrafish embryogenesis when takes place in a medium containing a dosage of 1000 microM zinc resulted in high mortality, indicating the deleterious effect of zinc on development. The zebrafish mt gene consists of three exons encoding 60 amino acids with 20 conserved cysteine residues. RT-PCR result indicates the maternal contribution of Mt transcripts. Using digoxigenin (DIG)-labeled anti-sense RNA probe, whole-mount in situ hybridization was performed to observe the expression pattern of zebrafish mt gene during embryonic and early larval stages. Stronger as well as ubiquitous expression of mt gene during early embryonic stages narrowed to specific expression after hatching. The mt promoter region contains seven copies of putative metal-responsive elements (MREs), which are shown to be important for the high level activity by deletion analysis. The expression of mt gene during embryogenesis implies its significant role on development.

Cheung, et al. (2005). "Tilapia metallothionein genes: PCR-cloning and gene expression studies." Biochim Biophys Acta 1731(3): 191-201.

Genomic PCR reactions were performed to isolate gene sequences of tilapia metallothionein (tiMT) from Oreochromis mossambicus and Oreochromis aureus. Two AP1 binding sites, four metal responsive elements, and a TATA box are the major cis-acting elements identified in the 800-bp 5' flanking region of the tiMTs obtained in this study. The tiMT gene promoter cloned from O. aureus was characterized in vitro using PLHC-1 cell-line, a hepatocellular carcinoma of a desert topminnow (Poecciliopsis lucida), following the administrations of Cd2+, Co2+, Cu2+, Ni2+, Pb2+ and Zn2+. Only Cd2+, Pb2+ and Zn2+ were able to induce the transcription of tiMT gene promoter in PLHC-1 cells in a dose-dependent manner. Zn2+ had the highest fold induction of tiMT gene promoter activity. Deletion mutants were tested for their abilities to drive the transcription of reporter gene following Cd2+ and Zn2+ administrations. However, Cu2+ and Ni2+ also induced the production of hepatic MT mRNA in vivo. Northern blot analysis showed that liver gave the highest fold induction of MT gene expression following the administration of heavy metal ions. These data indicated that hepatic MT mRNA level in tilapia is a potential sensitive biomarker of exposure to various metal ions including Cu2+, Cd2+, Ni2+, Pb2+, Hg2+ and Zn2+ ions.

Cheung, A. P., et al. (2004). "Regulation of Tilapia metallothionein gene expression by heavy metal ions." Mar Environ Res 58(2-5): 389-394.

Tilapia is a common fish species inhabiting inland waters and estuarine regions in Hong Kong and Southeast Asia, and useful for bio-monitoring of metal pollution. Metallothionein (MT) gene expression in fish tissues has been useful to sub-lethal risk assessment as biomarker of exposure to metal ions in fishes inhabiting metal contaminated area. To investigate metal inductions of Tilapia MT gene expression in vivo, Tilapias were injected with different concentrations of heavy metals and tissues were then removed for quantitative PCR assay using mimic PCR methods. All of the metal ions tested (Cu(2+), Cd(2+), Hg(2+), Ni(2+), Pb(2+) and Zn(2+)) were able to induce hepatic MT mRNA levels. Renal MT mRNA levels of Cd(2+) and Zn(2+) treated fish was not induced with significant fold induction, however MT mRNA levels in gills were sensitive to the administrations of these metal ions. These data indicated that Tilapia MT mRNA levels in gills and liver are sensitive biomarker of exposure to various metal ions.

Cho, Y. S., et al. (2008). "Gene structure and expression of metallothionein during metal exposures in Hemibarbus mylodon." Ecotoxicol Environ Saf 71(1): 125-137.

Metallothionein gene was characterized in Hemibarbus mylodon, an endangered fish species. H. mylodon MT shared a high homology with other vertebrate MTs, including (1) tripartite exon/intron structure, (2) typical regulatory elements such as MREs and GC boxes in the 5'-flanking region, and (3) high proportion of Cysteines (33.3%) in its amino acid sequence. MT mRNA was ubiquitously detected in various tissues. Basal level of MT mRNA was the highest in ovary while the lowest in heart. Transcription of MT was highly inducible by exposures to waterborne cadmium (0.1-10 microM), copper (2-10

microM) or zinc (2-10 microM), based on real-time RT-PCR. Cadmium was more potent for the stimulation of MT transcripts than copper and zinc. Liver was more responsive to heavy metals than kidney and gill. In overall, the transcriptional activation of MT gene by metal exposures followed a dose- and/or time-dependent fashion.

Cho, Y. S., et al. (2009). "Two metallothionein genes from mud loach Misgurnus mizolepis (Teleostei; Cypriniformes): gene structure, genomic organization, and mRNA expression analysis." Comp Biochem Physiol B Biochem Mol Biol 153(4): 317-326.

Two metallothionein genes, MLMT-IA and MLMT-IB, were isolated and characterized from the mud loach Misgurnus mizolepis (Teleostei; Cypriniformes). For these MTs, we determined a tandem "tail-to-head" genomic organizational pattern, identified conserved genomic features, showed high sequence identities in the coding regions, and examined the closest phylogenetic affiliation, suggesting their divergence by a recent gene duplication event. However, the 5'-flanking upstream regions in MLMT-IA and MLMT-IB exposed large differences in the composition and distribution patterns of various transcription factor binding motifs, especially regarding the organization of the metal response element clusters. Real-time RT-PCR assays showed that mRNA levels of both MLMT-IA and MLMT-IB isoforms were variable among tissues and the ratios between them were also variable across tissues, although the MLMT-IA was always predominant in every adult tissue tested. We also found that the MLMT-IA and MLMT-IB mRNA expression levels were regulated dynamically during embryonic and larval development stages, in which the basal expression level of MLMT-IA was also consistently higher than that of MLMT-IB. Upon acute in vivo metal exposure to cadmium, chromium, copper, iron, manganese, nickel, or zinc at 5 microM for 48 h, the transcriptional modulations of MLMT-IA and MLMT-IB were quite different from each other and the type of response was affected significantly by the kind of metals and tissues.

Choi, D., et al. (1996). "Molecular cloning of a metallothionein-like gene from Nicotiana glutinosa L. and its induction by wounding and tobacco mosaic virus infection." Plant Physiol 112(1): 353-359.

The cloning and characterization of genes expressed in plant disease resistance could be an initial step toward understanding the molecular mechanisms of disease resistance. A metallothionein-like gene that is inducible by tobacco mosaic virus and by wounding was cloned in the process of subtractive cloning of disease resistance-response genes in Nicotiana glutinosa. One 530-bp cDNA clone (KC9-10) containing an open reading frame of 81 amino acids was characterized. Genomic Southern blot hybridization with the cDNA probe revealed that tobacco metallothionein-like genes are present in few or in one copy per diploid genome. Northern blot hybridization detected strong induction of a 0.5-kb mRNA by wounding and tobacco mosaic virus infection, but only mild induction was detected when copper was tested as an inducer. Methyl jasmonate, salicylic acid, and ethylene were also tested as possible inducers of this gene, but they had no effect on its expression. The possible role of this gene in wounded and pathogen-stressed plants is discussed.

Choudhuri, S., et al. (1993). "Differential expression of the metallothionein gene in liver and brain of mice and rats." Toxicol Appl Pharmacol 119(1): 1-10.

Expression of the metallothionein I (MT-I) gene was studied in liver and brain of control mice and rats, as well as following administration of Cd and lipopolysaccharide (LPS). Time-course studies revealed that MT mRNA reached a maximum in liver of both mice and rats 6 hr following treatment with Cd or LPS. MT mRNA from control and Cd- and LPS-treated rat brains could not be detected by Northern-blot analysis of total RNA, but Northern analysis with poly(A)-enriched RNA revealed that induction of MT mRNA in rat brain does occur with both Cd and LPS treatment. In contrast, mouse brain MT mRNA was easily detected by Northern-blot analysis of total RNA. It was also clear from Northern-blot analyses of both mouse and rat brain that LPS induced more MT mRNA than did Cd. Quantitation of MT mRNA by solution hybridization revealed that Cd and LPS induced similar amounts of MT mRNA in livers of mice (about 0.64 fmol/micrograms total RNA by Cd and 0.68 by LPS) and rats (about 0.23 fmol/micrograms total RNA by Cd and 0.21 by LPS). Therefore, both inducers increased MT mRNA about threefold more in mouse liver than in rat liver. In mouse and rat brain, LPS induced about twice as much MT mRNA as did Cd (about 0.08 fmol/micrograms total RNA by Cd and 0.16 by LPS in mice and about 0.006 fmol/micrograms total RNA by Cd and 0.008 by LPS in rats). However, the actual amount of MT mRNA induced in rat brain by either inducer was minimal compared to that in mouse brain. In fact, Cd induced 13 times more MT mRNA in mouse brain than in rat brain, and LPS induced about 20 times more MT mRNA in mouse brain than in rat brain. Cd distribution to liver was similar in both mice and rats, but the Cd concentration in mouse brain was about 60% more than that in rat brain. Distribution of LPS was also similar in mouse and rat livers, as well as in mouse and rat brains. Therefore, there exists a difference in the expression of MT gene in both liver and brain of mice and rats, the expression in mice being higher than that in rats. These findings suggest that such differential expression of the MT gene cannot be entirely accounted for by the difference in the tissue

distribution of inducers. Other tissue-specific and species-specific factors controlling MT gene expression appear to be involved.

Chu, A., et al. (2015). "Zinc-induced upregulation of metallothionein (MT)-2A is predicted by gene expression of zinc transporters in healthy adults." Genes Nutr 10(6): 44.

The usefulness of zinc transporter and metallothionein (MT) gene expressions to detect changes in zinc intake remains unclear. This pilot study aimed to determine the effects of zinc supplementation on zinc transporter and MT gene expressions in humans. Healthy adults (n = 39) were randomised to zinc treatment (ZT), receiving 22 mg Zn/day (n = 19), or no treatment (NT) (n = 20). Blood samples were collected on Days 0, 2, 7, 14, and 21. Plasma zinc and serum C-reactive protein concentrations were analysed. Gene expression of zinc transporters and MT in peripheral blood mononuclear cells was analysed using real-time PCR. Using repeated-measures ANOVA, MT-2A gene expression and fold change were found to be higher in the ZT group (P = 0.025 and P = 0.016, respectively) compared to the NT group, specifically at Day 2 (40 +/- 18 % increase from baseline, P = 0.011), despite no significant increase in plasma zinc concentration. In a multiple regression model exploring the changes in gene expressions between Days 0 and 21, the change in MT-2A gene expression was correlated with changes in all zinc transporter expressions (r (2) = 0.54, P = 0.029); the change in ZIP1 expression emerged as a univariate predictor (P = 0.003). Dietary zinc intake was predictive of zinc transporter and MT expressions (P = 0.030). Physical activity level was positively correlated with baseline ZIP7 expression (r = 0.36, P = 0.029). The present study shows that MT-2A expression is related to changing expression of zinc transporter genes, specifically ZIP1, in response to zinc supplementation. The current report adds to our understanding of MT in the coordinated nature of cellular zinc homeostasis.

Chu, C. P., et al. (2005). "Enhanced cardiovascular alteration and Fos expression induced by central salt loading in a conscious rat transgenic for the metallothionein-vasopressin fusion gene." Neurosci Res 53(2): 147-155.

The present study is an investigation of the responses of the cardiovascular system and Fos expression to intracerebroventricular (i.c.v.) administration of hypertonic saline (HS) in conscious arginine vasopressin (AVP)-overexpressing transgenic (Tg) and control rats. Central HS (0.3, 0.67, or 1.0M NaCl, 1 microl/min for 20 min) significantly increased the mean arterial blood pressure (MABP) and Fos-like immunoreactivity (FLI) in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus, the area postrema (AP), the median preoptic nucleus (MnPO), and the organum vasculosum laminae terminalis (OVLT) in both Tg and control rats. The changes in MABP and FLI were significantly larger in Tg rats than in control rats. i.c.v. pretreatment with the AVP V1 receptor antagonist, OPC-21268, blocked the increase in MABP and significantly decreased the Fos expression in the PVN (posterior magnocellular (pm) component) induced by 0.3 M HS in the Tg rats. The present study demonstrates an increased responsiveness to i.c.v. administration of HS in AVP Tg rats, suggesting the relationship between the vasopressinergic drive and central cardiovascular response via, at least in part, the V1 receptor in the PVN magnocellular neurons.

Cicatelli, A., et al. (2010). "Arbuscular mycorrhizal fungi restore normal growth in a white poplar clone grown on heavy metal-contaminated soil, and this is associated with upregulation of foliar metallothionein and polyamine biosynthetic gene expression." Ann Bot 106(5): 791-802.

BACKGROUND AND AIMS: It is increasingly evident that plant tolerance to stress is improved by mycorrhiza. Thus, suitable plant-fungus combinations may also contribute to the success of phytoremediation of heavy metal (HM)-polluted soil. Metallothioneins (MTs) and polyamines (PAs) are implicated in the response to HM stress in several plant species, but whether the response is modulated by arbuscular mycorrhizal fungi (AMF) remains to be clarified. The aim of the present study was to check whether colonization by AMF could modify growth, metal uptake/translocation, and MT and PA gene expression levels in white poplar cuttings grown on HM-contaminated soil, and to compare this with plants grown on non-contaminated soil. METHODS: In this greenhouse study, plants of a Populus alba clone were pre-inoculated, or not, with either Glomus mosseae or G. intraradices and then grown in pots containing either soil collected from a multimetal- (Cu and Zn) polluted site or non-polluted soil. The expression of MT and PA biosynthetic genes was analysed in leaves using quantitative reverse transcription-PCR. Free and conjugated foliar PA concentrations were determined in parallel. RESULTS: On polluted soil, AMF restored plant biomass despite higher Cu and Zn accumulation in plant organs, especially roots. Inoculation with the AMF caused an overall induction of PaMT1, PaMT2, PaMT3, PaSPDS1, PaSPDS2 and PaADC gene expression, together with increased free and conjugated PA levels, in plants grown on polluted soil, but not in those grown on non-polluted soil. CONCLUSIONS: Mycorrhizal plants of P. alba clone AL35 exhibit increased capacity for stabilization of soil HMs, together with improved growth. Their enhanced stress tolerance may derive from the transcriptional upregulation of several stress-related genes, and the

protective role of PAs.

Cigliano, S., et al. (1996). "Analysis of metal-regulated metallothionein and heat shock gene expression in HeLa-derived cadmium-resistant cells." Exp Cell Res 228(2): 173-180.

The expression of metallothionein (MT) and heat shock protein gene families was investigated in normal and in HeLa-derived cadmium-resistant cells, named H454. In the absence of amplification of MT genes H454 cells accumulated elevated concentrations of cadmium ions and synthesized higher levels of MT proteins than unselected HeLa cells. Northern blot analyses revealed higher levels of MT mRNAs in the resistant cells than in wild-type cells after Cd2+ and Zn2+ exposure. Evaluation of the cytotoxic potential of the different metals confirmed the high resistance to cadmium of the H454 cells. Two proteins of the heat shock family, hsp70 and GRP78, were synthesized in Cd(2+)-exposed H454 cells at levels comparable to the ones present in Cd(2+)-treated normal cells. Northern blot analyses of the mRNA levels corresponding to these proteins revealed elevated expression of both hsp70 and GRP78 mRNAs in H454 cells upon exposure to cadmium ions and no response to zinc induction. These data suggest the existence in the H454 cells of a cadmium-specific pathway of regulation of MT and heat shock genes.

Compere, S. J. and R. D. Palmiter (1981). "DNA methylation controls the inducibility of the mouse metallothionein-I gene lymphoid cells." Cell 25(1): 233-240.

The W7 mouse thymoma cell line does not express the metallothionein-I (MT-I) gene in the presence of either cadmium or glucocorticoids, unlike most other cell lines. This cell line was therefore used as a model system for studying the role of DNA methylation on MT-I gene expression. The extent of DNA methylation within the MT-I gene and its flanking regions was determined by comparing the cleavage patterns generated by the isoschizomeric restriction enzymes Hpa II and Msp I. In W7 cells, all of the Hpa II sites in the vicinity of the MT-I gene are methylated, whereas in cells that have an expressible MT-I gene (for example, Friend erythroleukemia cells) all of these Hpa II sites are unmethylated. When W7 cells are treated for a few hours with 5-azacytidine, the MT-I gene becomes inducible by both cadmium and glucocorticoids. Addition of hydroxyurea along with 5-azacytidine prevents MT-I gene induction, suggesting that incorporation of 5-azacytidine into DNA is required before this gene can be activated. To determine whether 5-azacytidine treatment changes the methylation pattern near the MT-I gene, we treated W7 cells with 5-azacytidine and selected inducible cells in 10 micro M cadmium. all of the Hpa II sites within the MT-I gene are unmethylated in these cadmium-resistant W7 cells. In addition, flanking DNA sequences are also undermethylated in a pattern similar to that seen in Friend erythroleukemia cells that express the MT-I gene. The possible significance of methylation as a mechanism of gene commitment during cell differentiation is discussed.

Cong, M., et al. (2012). "Effects of heavy metals on the expression of a zinc-inducible metallothionein-III gene and antioxidant enzyme activities in Crassostrea gigas." Ecotoxicology 21(7): 1928-1936.

Sequestration by metallothioneins and antioxidant defense are two kinds of important defense mechanisms employed by mollusks to minimize adverse effects caused by heavy metal contaminants in marine environment. In the present study, a novel metallothionein gene, CgMT-III, was cloned from Crassostrea gigas, consisting of eighteen conserved cysteine residues and encoding a MT III-like protein with two tandem beta domains. The expression level of CgMT-III transcript induced by zinc was much higher than that induced by cadmium exposure. It suggested that CgMT-III was perhaps mainly involved in homeostatic control of zinc metabolism, which was distinct from previously identified MTs in C. gigas. Among the tested antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), SOD and GPx showed varying up-regulations in a tissue-specific manner, while CAT activities were inhibited in both gill and hepatopancreas from C. gigas exposed to heavy metals. It can be inferred that CgMT-III was mainly involved in zinc homeostasis, and CgMT-III gene together with CAT enzyme could be potential biomarkers to indicate heavy metal, especially zinc pollution in marine organisms.

Coogan, T. P., et al. (1994). "Enhanced metallothionein gene expression is associated with protection from cadmium-induced genotoxicity in cultured rat liver cells." J Toxicol Environ Health 41(2): 233-245.

Metallothioneins (MTs) are low-molecular-weight, cysteine-rich proteins that appear to play an important role in the cellular defense system against cadmium toxicity. Although substantial evidence exists demonstrating a reduction in cadmium toxicity concomitant with MT induction, little is known about the possible effects of stimulation of MT synthesis on cadmium-induced genotoxicity. Thus, the alkaline elution technique was used to assess single-strand DNA damage (SSD) in TRL-1215 cells, a liver-derived cell line shown to have inducible MT gene expression. The SSD accumulated over a 2-h time period in a time-dependent manner following exposure to 500 microM CdCl2. Low-concentration cadmium pretreatment (10 microM CdCl2, 24 h) provided protection against the genotoxicity of high-concentration cadmium (500 microM CdCl2, 2 h). A 2-h exposure to 500 microM

CdCl2 had no effect on viability, as assessed using a tetrazolium-dye based assay, in cells from either the pretreated or nonpretreated group. Metallothionein was induced in a time-dependent manner by low-concentration cadmium pretreatment: Exposure for 24 and 48 h resulted in 3.3- and 6.4-fold increases, respectively. In addition, a 24-h exposure to low-concentration cadmium resulted in an increase in MT-I gene expression. Cadmium accumulation was 2.6-fold greater in low-concentration cadmium-pretreated cells as compared to nonpretreated cells. These data demonstrate that low-concentration cadmium pretreatment provides protection against cadmium-induced single-strand DNA damage and support the hypothesis that this protection is due to stimulation of MT gene expression.

Coogan, T. P., et al. (1994). "Apparent quiescence of the metallothionein gene in the rat ventral prostate: association with cadmium-induced prostate tumors in rats." Environ Health Perspect 102 Suppl 3: 137-139.

Several chronic studies in rats indicating that cadmium exposure can induce tumors of the ventral prostate have recently been completed in our laboratory. In one such study, a single dose of cadmium, s.c., increased prostatic tumor incidence only at doses below 5.0 mumol/kg, the approximate threshold for cadmium-induced testicular damage. In a further study, prostatic tumors were elevated with higher doses of cadmium (30 mumol/kg, s.c.) if testicular damage was prevented by zinc pretreatment. Most recently, we found that dietary cadmium (25 to 200 micrograms/g) also can increase prostatic neoplastic lesions, but these were reduced by zinc-deficient diets. Thus it appears that cadmium produces prostatic tumors only if testicular function is maintained. Furthermore, we find that metallothionein (MT), a protein associated with cadmium tolerance, may be deficient in the rat prostate, and the prostatic MT gene, at least in the ventral lobe, is unresponsive to metal stimuli. In liver, MT gene expression, as assessed by MT-1 mRNA, was quite apparent in control tissue and was induced in a dose-dependent manner 24 hr following cadmium exposure (1 to 10 mumol/kg, s.c.). However, in the ventral prostate very low constitutive levels of MT-1 mRNA were detected and increases did not occur with cadmium exposure. Cadmium concentrations in the ventral prostate were in excess of those that cause significant induction in the liver. In sharp contrast to the gene in the ventral prostate, in the dorsal prostate the MT gene was quite active. The dorsal prostate is not susceptible to cadmium carcinogenesis.(ABSTRACT TRUNCATED AT 250 WORDS)

Coogan, T. P., et al. (1995). "Minimal basal activity and lack of metal-induced activation of the metallothionein gene correlates with lobe-specific sensitivity to the carcinogenic effects of cadmium in the rat prostate." Toxicol Appl Pharmacol 132(1): 164-173.

Metallothionein (MT), a high-affinity metal-binding protein, is known to detoxicate cadmium and may play an important role in cadmium carcinogenesis. In the rat, the ventral lobe of the prostate is sensitive to cadmium carcinogenesis, while the dorsolateral lobe is refractory. The possibility exists that the basis of this lobe-specific sensitivity may lie in the expression of the MT gene. Thus, the expression of the MT gene in lobes of the rat prostate was studied and, for comparative purposes, the expression of the MT gene in the liver, a tissue with well-defined high activity, was also assessed. MT gene expression was determined using a cDNA probe specific for MT-I, oligonucleotide probes specific for MT-I and MT-II, and an assay for cadmium-binding protein capacity. Basal levels of MT-I mRNA and cadmium-binding protein were much less in the ventral prostate than in the liver or dorsolateral prostate. Cadmium, given at a dose known to induce tumors of the ventral prostate (2.5 mumol/kg, sc), did not result in an increase in MT gene expression in the ventral prostate, as assessed by cadmium-binding protein levels or MT-I mRNA, over 72 hr. Small elevations of cadmium-binding protein capacity were detected at high doses of cadmium (25 and 40 mumol/kg) in the ventral prostate but no corresponding increases in MT mRNA were seen. In sharp contrast, hepatic MT gene expression was highly activated throughout the dosage range. Dose-response analysis 24 hr after cadmium administration (0.25 to 40 mumol/kg, sc) showed that MT-I and MT-II mRNA levels were increased in liver in a dose-dependent manner, while no evidence was found for MT gene activation in ventral prostate. In the dorsolateral prostate the high basal activity of the MT gene was shown, as assessed by MT-I and MT-II mRNA levels, which was not further elevated by cadmium treatments. Cadmium accumulation was much lower in the ventral prostate than in the liver. However, levels of cadmium that were sufficient to activate the hepatic MT gene had, in fact, reached the ventral prostate. Thus, the poor basal expression and lack of activation of the MT gene within the ventral lobe of the rat prostate may be the genetic basis to this tissue's sensitivity to the carcinogenic effects of cadmium.

Cosma, G., et al. (1992). "Rat lung metallothionein and heme oxygenase gene expression following ozone and zinc oxide exposure." Toxicol Appl Pharmacol 117(1): 75-80.

We have conducted exposures in rats to determine pulmonary responses following inhalation of two common components of welding fumes, zinc oxide and ozone. To examine their effects on target-inducible gene expression, we measured mRNA levels of two metal-responsive genes, metallothionein (MT) and

heme oxygenase (HO), in lung tissue by RNA slot-blot analysis. A 3-hr exposure to ZnO fume via a combustion furnace caused a substantial elevation in lung MT mRNA at all concentrations tested. Exposures to 5 and 2.5 mg/m3 ZnO resulted in peak 8-fold increases in MT mRNA levels (compared to air-exposed control animal values) immediately after exposure, while 1 mg/m3 ZnO exposure caused a 3.5-fold elevation in MT mRNA. These levels returned to approximate control gene expression values 24 hr after exposure. In addition, ZnO exposure caused an immediate elevation in lung HO gene expression levels, with 8-, 11-, and 5-fold increases observed after the same ZnO exposure levels ($p < 0.05$). Like MT gene induction, HO mRNA values returned to approximate control levels 24 hr after exposure. In striking contrast to the induction of MT and HO gene expression after ZnO exposures, there was no elevation in gene expression following a 6-hr exposure to 0.5 and 1 ppm ozone, even when lungs were examined as late as 72 hr after exposure. Our results demonstrate the induction of target gene expression following the inhalation of ZnO at concentrations equal to, and below, the current recommended threshold limit value of 5 mg/m3 ZnO. Furthermore, the lack of effect of ozone exposure on MT and HO gene expression suggests no involvement of these genes in the acute respiratory response to this oxidant compound.

Cosma, G. N., et al. (1991). "Detection of cadmium exposure in rats by induction of lymphocyte metallothionein gene expression." J Toxicol Environ Health 34(1): 39-49.

The induction of metallothionein (MT) gene expression in lymphocytes of rats was determined in order to detect exposure in vivo to cadmium. Both acute and chronic CdCl2 exposures resulted in the induction of the MT-1 gene in lymphocytes as measured by standard RNA Northern blot analysis. Twenty-four hours following an ip injection of 3.4 mg/kg CdCl2, a ninefold increase in MT gene expression was observed in lymphocytes, as well as five- and sevenfold increases in liver and kidney, respectively. Oral exposure of rats to 1-100 ppm CdCl2 via drinking water resulted in an approximate twofold enhanced MT signal in lymphocytes after 6 wk, and a threefold increase after 13 wk of exposure to 100 ppm Cd. No increases in lymphocyte MT gene expression were observed after 3 wk of Cd exposure. Liver MT gene expression was substantially induced following chronic Cd exposure, while kidney was not, although this organ had a higher basal expression of the MT-1 gene. Analysis of tissue Cd burdens demonstrated a dose-response Cd accumulation in liver and kidney, but only kidney burdens increased substantially with prolonged Cd exposure. These results demonstrate the utility of lymphocyte gene expression assays to detect in vivo toxicant exposure, and thus their applicability as molecular biomarker assays for human exposure assessment.

Cousins, R. J., et al. (1986). "Coordinate regulation of zinc metabolism and metallothionein gene expression in rats." Am J Physiol 251(6 Pt 1): E688-694.

Regulation of zinc metabolism by dibutyryl cAMP, glucagon, and epinephrine was examined in rats fed adequate amounts of zinc. Dibutyryl cAMP, epinephrine, and glucagon each produced an increase in liver metallothionein levels by 10 h after they were first administered. The increase in liver metallothionein was inversely related to the serum zinc concentration. Treatment with dexamethasone, a glucocorticoid, accentuated these effects to some extent. Both metallothionein I and II were induced by dibutyryl cAMP and glucagon. Levels of metallothionein mRNA in total liver RNA extracts were measured by dot blot hybridization using a synthetic 21-base oligonucleotide complimentary to the 5' region of both the metallothionein I and II genes. Individual administration of dibutyryl cAMP, glucagon, and epinephrine increased the number of metallothionein mRNA molecules per cell by up to fourfold. The data suggest that glucagon and epinephrine are primary regulators of metallothionein gene expression acting at least in part via cAMP. In adrenalectomized rats, glucagon, dibutyryl cAMP, and epinephrine had a less potent effect in terms of metallothionein induction and depression of serum zinc concentrations. These effects were largely restored when dexamethasone was also given. Collectively these data suggest that changes in zinc metabolism associated with acute stress involve coordinate regulation mediated by many factors, including glucocorticoids and cAMP.

Cousins, R. J. and L. M. Lee-Ambrose (1992). "Nuclear zinc uptake and interactions and metallothionein gene expression are influenced by dietary zinc in rats." J Nutr 122(1): 56-64.

Regulation of metallothionein gene expression by dietary zinc and the relationship of dietary zinc to nuclear zinc uptake was examined in growing rats. Zinc was fed at 5, 30 or 180 mg/kg, either in pelleted form for a 2-wk period (ad libitum) or for 2 h as a liquefied preparation (1 g in 4 mL). Two hours after the oral dose, the intestine and liver took up more zinc than other tissues. Nuclei purified from liver, kidney and spleen accumulated substantial amounts of zinc and directly reflected the dietary zinc level within the 2-h feeding period. Nuclei from kidney accumulated the largest amount of dietary zinc within 2 h, accounting for up to 6.2% of the total nuclear zinc concentration. Northern analysis demonstrated that metallothionein expression was proportional to dietary zinc intake in some tissues. It was greatest in kidney, followed in descending order by liver, intestine, spleen and heart.

Thymus and lung metallothionein mRNA levels were not changed appreciably by dietary zinc intake. Chromatography of extracts from liver nuclei shows that 65Zn introduced into the portal supply is bound to discrete fractions of nuclear proteins. One of these fractions binds both 65Zn and a 32P-labeled oligonucleotide corresponding to the metal regulatory element of the metallothionein promoter. These results demonstrate that significant amounts of zinc from the diet are rapidly taken up by cell nuclei. Furthermore, they suggest that transcriptional regulation of the metallothionein gene and other genes with metal regulatory elements involves a direct interaction between the dietary supply and intranuclear factors that bind zinc.

Cox, D. R. and R. D. Palmiter (1983). "The metallothionein-I gene maps to mouse chromosome 8: implications for human Menkes' disease." Hum Genet 64(1): 61-64.

We have assigned the structural gene (Mt-1) coding for the murine metal-binding protein metallothionein I (MT-1) to mouse chromosome 8 by using a cloned DNA probe for mouse Mt-1 in combination with a panel of Chinese hamster-mouse somatic cell hybrid clones segregating mouse chromosomes. Analysis of hybrid cell extracts for the presence of mouse Mt-1 or MT-1 mRNA revealed concordant segregation of Mt-1 with mouse glutathione reductase, an enzyme marker for mouse chromosome 8, but discordant segregation with enzyme markers for 14 other mouse chromosomes. Karyotype analyses of seven informative hybrid clones confirmed the assignment of mouse Mt-1 to chromosome 8. Menkes' disease in man and the mottled mutation (Mo) in the mouse, which provides an animal model of Menkes' disease, are both X-linked degenerative neurologic disorders involving abnormal copper metabolism and increased levels of intracellular metallothionein protein. Fibroblasts from Mo male mice have increased amounts of MT-1 mRNA, suggesting that both Mo and Menkes' disease may be due to a metallothionein gene mutation. However, our assignment of Mt-1 to mouse chromosome 8, rather than the X chromosome, demonstrates that a mutation in mouse Mt-1 or a closely linked regulatory gene is not the primary defect in Mo, and implies that a metallothionein gene mutation is not the genetic defect in human Menkes' disease.

Cozza, R., et al. (2013). "Expression pattern of a type-2 metallothionein gene in a wild population of the psammophyte Silene nicaeensis." Protoplasma 250(1): 381-389.

Silene nicaeensis is a wild Mediterranean grass often restricted to sandy sea shore and exhibiting an excellent tolerance to drought and salinity. Within Silene genus, several heavy metal-tolerant ecotypes have been identified, but information on molecular basis of such metal tolerance is still limited. Conceivably, salt-tolerant plants may represent a powerful tool for the remediation of heavy metal contaminated sites in saline environment. Here, a gene encoding a metallothionein protein was isolated from S. nicaeensis. Sequence analysis identified the motifs characteristic of type II metallothionein and designated as SnMT2. SnMT2 expression was investigated in plants collected from two sites differing in Metal Pollution Index (MPI). SnMT2 expression by polymerase chain reaction-based semi-quantitative transcript analysis showed a high accumulation in the leaves; in situ hybridization showed a steady localization of SnMT2 mRNA in the vascular bundle and in proliferating tissues. Moreover, an increase of SnMT2 was observed in the root of plants collected from area with higher MPI. The putative role of SnMT2 in metal tolerance is discussed.

Crawford, B. D., et al. (1985). "Coordinate amplification of metallothionein I and II genes in cadmium-resistant Chinese hamster cells: implications for mechanisms regulating metallothionein gene expression." Mol Cell Biol 5(2): 320-329.

We describe here the derivation, characterization, and use of clonal cadmium-resistant (Cdr) strains of the Chinese hamster cell line CHO which differ in their metallothionein (MT) induction capacity. By nondenaturing polyacrylamide gel electrophoresis, we showed that the stable Cdr phenotype is correlated with the augmented expression of both isometallothioneins (MTI and MTII). In cells resistant to concentrations of CdCl2 exceeding 20 microM, coordinate amplification of genes encoding both isometallothioneins was demonstrated by using cDNA MT-coding sequence probes and probes specific for 3'-noncoding regions of Chinese hamster MTI and MTII genes. Molecular and in situ hybridization analyses supported close linkage of Chinese hamster MTI and MTII genes, which we have mapped previously to Chinese hamster chromosome 3. This suggests the existence of a functionally related MT gene cluster in this species. Amplified Cdr variants expressing abundant MT and their corresponding Cds parental CHO cells should be useful for future studies directed toward elucidating the mechanisms that regulate expression of the isometallothioneins.

Crenshaw, E. B., 3rd, et al. (1987). "Neuron-specific alternative RNA processing in transgenic mice expressing a metallothionein-calcitonin fusion gene." Cell 49(3): 389-398.

Alternative RNA processing of the calcitonin/CGRP gene generates transcripts encoding predominantly calcitonin in thyroid C cells or CGRP in the nervous system. To examine the RNA processing choice of this gene in a wide variety of tissues, we

created transgenic mice expressing the rat calcitonin/CGRP transcript from the mouse metallothionein-I promoter. Most cells that do not express the endogenous calcitonin/CGRP gene have the capability to make a clear splicing choice for calcitonin or CGRP transcript. In the majority of tissues studied, 90%-97% of the transgene mRNA encodes calcitonin. In contrast, both calcitonin and CGRP mRNAs were detected in the transgenic mice brains. Immunohistochemical and in situ RNA hybridization analyses show that CGRP transcripts are selectively expressed in a wide variety of neurons, while calcitonin is expressed predominantly in nonneuronal structures. Splicing choice operates independently of calcitonin/CGRP gene transcription. The data suggest that a specific regulatory machinery is required for the processing of CGRP transcripts and is restricted primarily to neurons.

Cserjesi, P., et al. (1992). "Functional analysis of the promoter of a sea urchin metallothionein gene." Biochem Cell Biol 70(10-11): 1142-1150.

The 5'-flanking region of the metallothionein (MT) gene LpMT1 of the sea urchin Lytechinus pictus includes three copies of a conserved sequence that includes the metal-responsive element (MRE) consensus core sequence required for heavy metal induction of other MT genes, a GC box, a G box of a putative basal level enhancer element which includes another MRE core element, and a poly(C) tract. A fragment of LpMT1 DNA from nucleotides +31 to -309 fused to a chloramphenicol acetyltransferase reporter gene was inducible with cadmium after injection into L. pictus embryos. This induced activity was greatly reduced in a deletion mutant which retained only 195 base pairs of 5'-flanking sequence, including the proximal pair of MREs and the G box, but excluding the poly(C) tract, GC box, and distal MRE. A potent human hMT-IIA gene promoter is marginally functional in L. pictus embryos. In contrast, the LpMT1 promoter is active in HeLa cells and in embryos of the sea urchin Strongylocentrotus purpuratus. The hMT-IIA gene may lack a cis-acting sequence element required for expression of MT genes in L. pictus embryos. The LpMT1 promoter is a powerful, inducible, promiscuous promoter useful for driving the expression of heterologous genes in sea urchin embryos.

Cserjesi, P., et al. (1997). "Metallothionein gene expression in embryos of the sea urchin Lytechinus pictus." Mol Reprod Dev 47(1): 39-46.

The metallothionein (MT) gene LpMT1 of the sea urchin Lytechinus pictus was characterized. The primary transcript of 3042 nucleotides includes four exons, as uniquely observed for other sea urchin MT genes, which are spliced to form a messenger RNA of 605 nucleotides. The deduced LpMT1 protein sequence includes 69 amino acids, more than observed for other MT proteins. For a high level of inducible activity, the LpMT1 promoter requires sequence elements in addition to the canonical regulatory elements identified for mammalian MT promoters. The promoter of the closely related LpMT2 gene is very active in spite of its lack of a distinctive poly(C) element included in a sequence tract required for fully induced activity of the LpMT1 promoter. In contrast to embryos of the sea urchin S. purpuratus in which MT mRNAs are restricted to the aboral ectoderm of uninduced embryos, no spatially preferential accumulation of MT mRNAs in L. pictus embryos was observed. The cisacting regulatory elements required for MT gene activity and the spatial specificity of MT gene expression in sea urchin embryos are considered. The LpMT1 and LpMT2 promoters constitute promiscuous promoters that can be induced to a high level of activity.

Cui, Y., et al. (2003). "ECRG2, a novel candidate of tumor suppressor gene in the esophageal carcinoma, interacts directly with metallothionein 2A and links to apoptosis." Biochem Biophys Res Commun 302(4): 904-915.

Esophageal cancer related gene 2 (ECRG2) is a novel candidate of the tumor suppressor gene identified from human esophagus. To study the biological role of the ECRG2 gene, we performed a GAL4-based yeast two-hybrid screening of a human fetal liver cDNA library. Using the ECRG2 cDNA as bait, we identified nine putative clones as associated proteins. The interaction of ECRG2 and metallothionein 2A (MT2A) was confirmed by glutathione S-transferase pull-down assays in vitro and co-immunoprecipitation experiments in vivo. ECRG2 co-localized with MT2A mostly to nuclei and slightly to cytoplasm, as shown by confocal microscopy. Transfection of ECRG2 gene inhibited cell proliferation and induced apoptosis in esophageal cancer cells. In the co-transfection of ECRG2 and MT2A assays, cell proliferation was inhibited and apoptosis was slightly induced compared with control groups. When we used antisense MT2A to interdict the effect of MT2A, the inhibition of cell proliferation and induction of apoptosis were significantly enhanced. When we used antisense ECRG2 to interdict the effect of ECRG2 in the group of Bel7402 cells co-transfected with ECRG2 and MT2A, the inhibition of cell proliferation and induction of apoptosis disappeared. The results provide evidence for ECRG2 in esophageal cancer cells acting as a bifunctional protein associated with the regulation of cell proliferation and induction of apoptosis. ECRG2 might reduce the function of MT2A on the regulation of cell proliferation and induction of apoptosis. The physical interaction of ECRG2 and MT2A may play an important role in the carcinogenesis of esophageal cancer.

Culotta, V. C. and D. H. Hamer (1989). "Fine mapping of a mouse metallothionein gene metal response element." Mol Cell Biol 9(3): 1376-1380.

Metal-regulated transcription of metallothionein (MT) genes in higher eucaryotes involves multiple copies of a highly conserved 17-base-pair metal-regulatory element (MRE). We have assayed by transient transfection the ability of mouse MT-I element d (MREd) to confer metal responsivity to constructs containing the mouse MT-I TATA box and the bacterial chloramphenicol acetyltransferase indicator gene. A single copy of MREd works bidirectionally to afford a three- to fourfold induction, and dual copies act cooperatively to yield a 10- to 20-fold response. Element d responds to the same spectrum of heavy metals as doses the complete MT gene promoter. The sequences involved in induction by metals were delineated by analyzing point mutations in MREd. While nucleotides of the highly conserved core sequence TGCPuCXC are critical, substitutions in the less conserved regions affect the induction response only marginally. These sequences include residues of a potential Sp1-binding site, suggesting that if Sp1 binds to MREd, it has little if any role in induction by metals.

Culotta, V. C., et al. (1989). "Copper and the ACE1 regulatory protein reversibly induce yeast metallothionein gene transcription in a mouse extract." Proc Natl Acad Sci U S A 86(21): 8377-8381.

We describe a cell-free system in which the transcription of the yeast metallothionein gene is inducible by the addition of metal ions plus a specific regulatory protein. Efficient transcription requires the complete yeast ACE1 metalloregulatory protein, including both its DNA-binding and transactivation domains; a mouse nuclear extract providing RNA polymerase and general transcription factors; a template containing the ACE1 binding site; and Cu(I). Because the binding of ACE1 to DNA is dependent on Cu, it is possible to inhibit transcription by the use of Cu-complexing agents such as CN-. We have used this specific inhibition to show that the ACE1 regulatory protein is required for the maintenance as well as the formation of a functional preinitiation complex. The ability to reversibly induce yeast metallothionein gene transcription in vitro provides a powerful system for determining the molecular mechanism of a simple eukaryotic regulatory circuit.

Cwikel, B. J. and J. F. Habener (1987). "Provasopressin-neurophysin II processing is cell-specific in heterologous cell lines expressing a metallothionein-vasopressin fusion gene." J Biol Chem 262(29): 14235-14240.

Preprovasopressin-neurophysin II (prepro-AVP-Np), the precursor of the cyclic, amidated nonapeptide, arginine vasopressin (AVP), is present in the central and peripheral nervous systems, adrenal glands, and gonads of rats. To study cell-specific processing of prepro-AVP-Np, a fusion gene consisting of the heavy metal-inducible promoter of the mouse metallothionein I gene and the rat prepro-AVP-Np gene was introduced by cellular transfection into several defined cell phenotypes: a fibroblast line (BHK), a pituitary growth hormone and prolactin-producing cell line (GH4), a pituitary cell line that produces several amidated peptides (AtT-20), and an insulin-producing pancreatic islet line (RIN- 1046-38). Clonal cell lines were isolated and prepro-AVP-Np-specific transcripts were detected by Northern blot hybridization analyses. Fibroblast BHK and pituitary GH4 cells transfected with the fusion gene synthesized a polypeptide (Mr = 18,000) characteristic of the glycosylated precursor, pro-AVP-Np; in metal -treated cells, this protein was the major secreted cysteine-labeled polypeptide. Extracts of RIN-1046-38 and AtT-20 cells transfected with the fusion gene contained predominantly processed neurophysin and amidated arginine vasopressin, whereas extracts of BHK and GH4 cells contained mainly precursors of AVP and neurophysin. These observations indicate that the pathways involving specific post-translational processing of pro-AVP-Np are more efficiently utilized in the prohormone-producing AtT-20 and RIN-1046-38 cells than in GH4 and BHK cells that do not synthesize any recognized prohormones.

Dabholkar, M., et al. (2000). "Increased mRNA levels of xeroderma pigmentosum complementation group B (XPB) and Cockayne's syndrome complementation group B (CSB) without increased mRNA levels of multidrug-resistance gene (MDR1) or metallothionein-II (MT-II) in platinum-resistant human ovarian cancer tissues." Biochem Pharmacol 60(11): 1611-1619.

Tumor tissue specimens from human ovarian cancer patients were assessed for relative mRNA abundance levels of several genes thought to be involved in the development of in vitro drug resistance in this disease. Higher mRNA levels of Xeroderma pigmentosum group B (XPB), which links DNA repair with DNA transcription, and of Cockayne's syndrome group B (CSB), which is essential for gene-specific repair, were observed in tumor tissues that were clinically resistant to platinum-based chemotherapy, as compared with tissues from patients responding to therapy. In a cohort of 27 patients, mRNA levels of XPB averaged 5-fold higher in platinum-resistant tumors ($P = 0.001$); and for CSB, mRNA levels averaged 6-fold higher but with greater variability ($P = 0.033$). Concurrently, these platinum-resistant tumor tissues did not exhibit significantly higher mRNA levels for the MDR1 (multidrug-resistance) gene ($P = 0.134$) or of the metallothionein-II (MT-II) gene ($P = 0.598$). Since these platinum-resistant tumors also show higher mRNA levels of ERCC1 and XPA, platinum

resistance appears to be associated with concurrent up-regulation of four genes (XPA, ERCC1, XPB, and CSB). These four genes participate in DNA damage excision activity, gene-specific repair, and linkage of DNA repair with DNA transcription. These data suggest that concurrent up-regulation of genes involved in nucleotide excision repair may be important in clinical resistance to platinum-based chemotherapy in this disease.

Dalton, T., et al. (1994). "Transcriptional induction of the mouse metallothionein-I gene in hydrogen peroxide-treated Hepa cells involves a composite major late transcription factor/antioxidant response element and metal response promoter elements." Nucleic Acids Res 22(23): 5016-5023.

Synthesis of metallothionein-I (MT-I) and heme oxygenase mRNAs is rapidly and transiently induced by H2O2 in mouse hepatoma cells (Hepa) and this effect is blocked by catalase. Menadione, which generates free radicals, also induces these mRNAs. Deletion mutagenesis revealed that a region between -42 and -153 in the mouse MT-I promoter was essential for induction of a CAT reporter gene. A multimer of a 16 bp sequence (-101 to -86) that includes an antioxidant response element and overlapping adenovirus major late transcription factor binding site elevated basal expression and allowed induction by H2O2 when inserted upstream of a minimal promoter. However, deletion of this region (-100 to -89) from the intact MT-I promoter (-153) did not completely eliminate response. Multiple copies of a metal response element also permitted response to H2O2. These results suggest that induction of MT-I gene transcription by H2O2 is mediated by at least two different elements within the proximal MT-I gene promoter and suggest a previously undescribed function of the MRE. Induction of MT gene transcription by ROS and the subsequent scavenging of ROS by the MT peptide is reminiscent of the metal regulatory loop and is consistent with the hypothesized protective functions of MT.

Dalton, T. P., et al. (1996). "Oxidative stress activates metal-responsive transcription factor-1 binding activity. Occupancy in vivo of metal response elements in the metallothionein-I gene promoter." J Biol Chem 271(42): 26233-26241.

Oxidative stress (tert-butylhydroquinone) rapidly induced metallothionein-I gene expression in mouse Hepa cells, and this effect was mediated predominantly through metal response promoter elements in transient transfection assays. In vivo genomic footprinting of the mouse metallothionein-I promoter after treatment of Hepa cells with hydrogen peroxide, tert-butylhydroquinone, or zinc suggested a rapid increase in occupancy of the metal response elements. More subtle changes also occurred in the constitutive genomic footprint at the composite major late transcription factor/antioxidant response element. This element may, in part, mediate induction by hydrogen peroxide. Electrophoretic mobility shift assays demonstrated a rapid (30 min) increase in the DNA binding activity of metal-responsive transcription factor-1 in Hepa cells treated with any of these inducers. In control cells, upstream stimulatory factor binding with the major late transcription factor site, and a nuclear protein complex distinct from AP-1, but specific for the antioxidant response element, were detected. The amounts of these complexes were not altered after these treatments. These studies indicate that metal-responsive transcription factor-1 plays a role in activating mouse metallothionein-I gene transcription in response to reactive oxygen species.

D'Anna, J. A. and R. A. Tobey (1989). "Changes in nucleosome repeat lengths precede replication in the early replicating metallothionein II gene region of cells synchronized in early S phase." Biochemistry 28(7): 2895-2902.

Previous investigations showed that inhibition of DNA synthesis by hydroxyurea, aphidicolin, or 5-fluorodeoxyuridine produced large changes in the composition and nucleosome repeat lengths of bulk chromatin. Here we report results of investigations to determine whether the changes in nucleosome repeat lengths might be localized in the initiated replicons, as postulated [D'Anna, J. A., & Prentice, D. A. (1983) Biochemistry 22, 5631-5640]. In most experiments, Chinese hamster (line CHO) cells were synchronized in G1, or they were synchronized in early S phase by allowing G1 cells to enter S phase in medium containing 1 mM hydroxyurea or 5 micrograms mL-1 aphidicolin, a procedure believed to produce an accumulation of initiated replicons that arise from normally early replicating DNA. Measurements of nucleosome repeat lengths of bulk chromatin, the early replicating unexpressed metallothionein II (MTII) gene region, and a later replicating repeated sequence indicate that the changes in repeat lengths occur preferentially in the early replicating MTII gene region as G1 cells enter and become synchronized in early S phase. During that time, the MTII gene region is not replicated nor is there any evidence for induction of MTII messenger RNA. Thus, the results are consistent with the hypothesis that changes in chromatin structure occur preferentially in the early replicating (presumably initiated) replicons at initiation or that changes in chromatin structure can precede replication during inhibition of DNA synthesis. The shortened repeat lengths that precede MTII replication are, potentially, reversible, because they become elongated when the synchronized early S-phase cells are released to resume cell cycle progression.

Dar, S., et al. (2013). "A synthetic cadmium

metallothionein gene (PMCd1syn) of Paramecium species: expression, purification and characteristics of metallothionein protein." Mol Biol Rep 40(2): 983-997.

Metallothioneins (MTs) are metal binding proteins that are rich in cysteine residues constituting 10-30 % of the total protein, and in which the thiol groups bind to the metal ions. The increasing amount of metal ions in the medium have shown increased production of MTs by different organisms such as bacteria, protozoa and mammals like humans. PMCd1 is the first gene ever discovered in Paramecium, a ciliated protozoan, that could produce this MT in response to cadmium. In this study the PMCd1syn gene has been cloned in pET41a expression vector and expressed in an Escherichia coli BL21-codonplus strain for the first time. Since the gene PMCd1 amplified from Paramecium contained 10 codons, which could act as stop codons during expression in E. coli, this gene of 612 bps was synthesized to substitute these (stop) codons for the Paramecium sp. specific amino acids. For stability of the expressed protein, glutathione-S-transferase gene was fused with PMCd1syn gene and coexpressed. The cells expressing PMCd1syn demonstrated increased accumulation of cadmium. This is the first report of cadmium MT protein expressed from Paramecium species, particularly from synthetic MT gene (PMCd1syn). This fusion protein, the molecular weight of which has been confirmed to be 53.03 kDa with MALDI analysis, is rich in cysteine residues, and has been shown for the first time in this ciliate to bind to and sequester Cd(2+)-ions.

Datta, P. K. and S. T. Jacob (1993). "Identification of a sequence within the mouse metallothionein-I gene promoter mediating its basal transcription and of a protein interacting with this element." Cell Mol Biol Res 39(5): 439-449.

Previous studies in our laboratory have shown that a trans-acting factor, which binds to a 106 bp sequence in the mouse metallothionein-I (MT-I) gene, is responsible for the relatively high level of MT-I gene transcription in the liver. Using electrophoretic mobility shift assay, we have now identified a 26 bp sequence within the 106 bp region, which interacts with a trans-activating factor in the liver nuclear extract. This sequence, designated MRE-c', is located between positions -135 and -110 with respect to the transcription start site and comprises the metal regulatory element MRE-c and part of its 5' and 3' flanking sequences. UV cross-linking and Southwestern analysis showed that a protein of an apparent molecular mass of 33,000 specifically interacts with MRE-c'. Deletion of the MRE-c' region resulted in a six- to sevenfold decrease in the MT-I promoter activity, as measured by reduction in chloramphenicol acetyltransferase activity. A comparison of other regulatory domains of the MT-I gene and the potential factors interacting with these sequences indicates that MRE-c' and probably the 33 kDa polypeptide are involved in the constitutive transcription of the MT-I gene.

Datta, P. K. and S. T. Jacob (1997). "Activation of the metallothionein-I gene promoter in response to cadmium and USF in vitro." Biochem Biophys Res Commun 230(1): 159-163.

To elucidate the molecular mechanism of metallothionein (MT) gene activation in response to various inducers, we constructed a G-less mouse MT-I promoter and transcribed in HeLa nuclear extract. The MT-I gene was transcribed efficiently in this extract and initiation of transcription occurred at the correct site (+1). Transcription of the MT-I gene was stimulated three- to fivefold in the nuclear extract from the cadmium-treated cells relative to the extract from the untreated cells. The MT-I promoter was also activated three- to fourfold by recombinant USF1, a helix-loop-helix-leucine zipper DNA binding transcription factor that recognizes the major late transcription factor (MLTF) binding site on the MT-I promoter. To our knowledge, this is the first report of the activation of MT-I promoter in vitro by a toxic metal and by the transcription factor USF.

Datta, P. K. and E. A. Lianos (2006). "Nitric oxide induces metallothionein-I gene expression in mesangial cells." Transl Res 148(4): 180-187.

In various forms of injury involving the renal glomerulus, mesangial cells are exposed to potentially toxic concentrations of nitric oxide (NO) caused by activation of the inducible isoform of nitric oxide synthase (NOS). Whether mesangial cells possess systems that can defend against NO mediated oxidative injury is unknown. One putative system is Metallothionein (MT). Metallothioneins constitute a family of cysteine proteins and play a significant role as anti-oxidants. The authors assessed whether NO upregulates MT-I expression in cultured glomerular mesangial cells. Northern blot analysis revealed that steady state MT-I mRNA levels were increased by three different NO donors: sodium nitroprusside (SNP), S-nitroso-N-acetyl-DL-penicillamine (SNAP), and Spermine-NONOate (Sper/NO). The increase in MT-I mRNA levels induced by SNAP-derived NO was attenuated by the antioxidant N-acetylcysteine (NAC), a glutathione (GSH) precursor, which indicates that the mechanism of NO-mediated MT-I expression may involve an oxidative stress response. These observations identify MT-I as a putative antioxidant system in NO-mediated mesangial cell injury.

David, E., et al. (2012). "Characterisation and genetic polymorphism of metallothionein gene CgMT4 in experimental families of Pacific oyster Crassostrea gigas displaying summer mortality." Biomarkers 17(1):

85-95.

Summer mortality events have been observed in Pacific oyster Crassostrea gigas for several decades. This paper examines the selective pressure exerted by summer mortality on the polymorphism of a newly identified oyster metallothionein gene. CgMT4 cDNA and genomic sequences were obtained. CgMT4 was studied in two generations of oysters reared in three sites on the French Atlantic coast, using single strand conformation polymorphism analysis. Four alleles were detected. Individuals carrying genotype MT4-CD seem to have higher susceptibility to summer risk conditions. The MT4 gene could be a potential new genetic marker for susceptibility; further validation studies are recommended.

de Framond, A. J. (1991). "A metallothionein-like gene from maize (Zea mays). Cloning and characterization." FEBS Lett 290(1-2): 103-106.

A differentially expressed maize gene has been cloned and sequenced. Transcriptional and translational start sites have been mapped and 2.5 kb of 5' flanking DNA were sequenced. The 8 kDa protein encoded by this gene shows striking similarity to the metallothionein-like proteins recently described in Pisum sativum and Mimulus guttatus. The maize MT-L gene message is very abundant in roots without exposure to high levels of metals, present at lower concentration in leaves and pith, and at very low concentration in seed.

de Francisco, P., et al. (2018). "AP-1 (bZIP) Transcription Factors as Potential Regulators of Metallothionein Gene Expression in Tetrahymena thermophila." Front Genet 9: 459.

Metallothioneins (MT) are multi-stress proteins mainly involved in metal detoxification. MT gene expression is normally induced by a broad variety of stimulus and its gene expression regulation mainly occurs at a transcriptional level. Conserved motifs in the Tetrahymena thermophila MT promoters have been described. These motifs show a consensus sequence very similar to AP-1 sites, and bZIP type transcription factors might participate in the MT gene expression regulation. In this research work, we characterize four AP-1 transcription factors in each of four different analyzed Tetrahymena species, detecting a high conservation among them. Each AP-1 molecule has its counterpart in the other three Tetrahymena species. A comparative qRT-PCR analysis of these AP-1 genes have been carried out in different T. thermophila strains (including metal-adapted, knockout and/or knockdown strains among others), and under different metal-stress conditions (1 or 24 h Cd(2+), Cu(2+), or Pb(2+) treatments). The possible interaction of these transcription factors with the conserved AP-1 motifs present in MT promoters has been corroborated by protein-DNA interaction experiments. Certain connection between the expression patterns of the bZIP and MT genes seems to exist. For the first time, and based on our findings, a possible gene expression regulation model including both AP-1 transcription factors and MT genes from the ciliate T. thermophila has been elaborated.

de Francisco, P., et al. (2017). "Extreme metal adapted, knockout and knockdown strains reveal a coordinated gene expression among different Tetrahymena thermophila metallothionein isoforms." PLoS One 12(12): e0189076.

Metallothioneins (MT) constitute a superfamily of small cytosolic proteins that are able to bind metal cations through numerous cysteine (Cys) residues. Like other organisms the ciliate Tetrahymena thermophila presents several MT isoforms, which have been classified into two subfamilies (Cd- and Cu-metallothioneins). The main aim of this study was to examine the specific functions and transcriptional regulation of the five MT isoforms present in T. thermophila, by using several strains of this ciliate. After a laboratory evolution experiment over more than two years, three different T. thermophila strains adapted to extreme metal stress (Cd2+, Cu2+ or Pb2+) were obtained. In addition, three knockout and/or knockdown strains for different metallothionein (MT) genes were generated. These strains were then analyzed for expression of the individual MT isoforms. Our results provide a strong basis for assigning differential roles to the set of MT isoforms. MTT1 appears to have a key role in adaptation to Cd. In contrast, MTT2/4 are crucial for Cu-adaptation and MTT5 appears to be important for Pb-adaptation and might be considered as an "alarm" MT gene for responding to metal stress. Moreover, results indicate that likely a coordinated transcriptional regulation exists between the MT genes, particularly among MTT1, MTT5 and MTT2/4. MTT5 appears to be an essential gene, a first such report in any organism of an essential MT gene.

de Francisco, P., et al. (2016). "The Tetrahymena metallothionein gene family: twenty-one new cDNAs, molecular characterization, phylogenetic study and comparative analysis of the gene expression under different abiotic stressors." BMC Genomics 17: 346.

BACKGROUND: Ciliate metallothioneins (MTs) are included in family 7 of the MT superfamily. This family has been divided into two main subfamilies: 7a or CdMTs and 7b or CuMTs. All ciliate MTs reported have been isolated from different Tetrahymena species and present unique features with regard to standard MTs. Likewise, an expression analysis has been carried out on some of MT genes under metal stress, corroborating their classification into two subfamilies. RESULTS: We isolated 21 new cDNAs from different Tetrahymena species to obtain a wider view of the biodiversity of these conserved genes.

Structural analysis (cysteine patterns) and an updated phylogenetic study both corroborated the previous classification into two subfamilies. A new CuMT from a Tetrahymena-related species Ichthyophthirius multifiliis was also included in this general analysis. We detected a certain tendency towards the presentation of a CdMT tri-modular structure in Borealis group species with respect to Australis group. We report for the first time a semi-complete paralog duplication of a CdMT gene originating a new CdMT gene isoform in T. malaccensis. An asymmetry of the codon usage for glutamine residues was detected between Cd- and CuMTs, and the phylogenetic implications are discussed. A comparative gene expression analysis of several MT genes by qRT-PCR revealed differential behavior among them under different abiotic stressors in the same Tetrahymena species. CONCLUSIONS: The Tetrahymena metallothionein family represents a quite conserved protein structure group with unique features with respect to standard MTs. Both Cd- and CuMT subfamilies present very defined and differentiated characteristics at several levels: cysteine patterns, modular structure, glutamine codon usage and gene expression under metal stress, among others. Gene duplication through evolution seems to be the major genetic mechanism for creating new MT gene isoforms and increasing their functional diversity.

De Oliveira, V. H., et al. (2020). "Bioremediation potential of Cd by transgenic yeast expressing a metallothionein gene from Populus trichocarpa." Ecotoxicol Environ Saf 202: 110917.

Cadmium (Cd) is an extremely toxic environmental pollutant with high mobility in soils, which can contaminate groundwater, increasing its risk of entering the food chain. Yeast biosorption can be a low-cost and effective method for removing Cd from contaminated aqueous solutions. We transformed wild-type Saccharomyces cerevisiae (WT) with two versions of a Populus trichocarpa gene (PtMT2b) coding for a metallothionein: one with the original sequence (PtMT2b 'C') and the other with a mutated sequence, with an amino acid substitution (C3Y, named here: PtMT2b 'Y'). WT and both transformed yeasts were grown under Cd stress, in agar (0; 10; 20; 50 muM Cd) and liquid medium (0; 10; 20 muM Cd). Yeast growth was assessed visually and by spectrometry OD600. Cd removal from contaminated media and intracellular accumulation were also quantified. PtMT2b 'Y' was also inserted into mutant strains: fet3fet4, zrt1zrt2 and smf1, and grown under Fe-, Zn- and Mn-deficient media, respectively. Yeast strains had similar growth under 0 muM, but differed under 20 muM Cd, the order of tolerance was: WT < PtMT2b 'C' < PtMT2b 'Y', the latter presenting 37% higher growth than the strain with PtMT2b 'C'. It also extracted ~80% of the Cd in solution, and had higher intracellular Cd than WT. Mutant yeasts carrying PtMT2b 'Y' had slightly higher growth in Mn- and Fe-deficient media than their non-transgenic counterparts, suggesting the transgenic protein may chelate these metals. S. cerevisiae carrying the altered poplar gene offers potential for bioremediation of Cd from wastewaters or other contaminated liquids.

De, S. K., et al. (1990). "Cadmium teratogenicity and its relationship with metallothionein gene expression in midgestation mouse embryos." Toxicology 64(1): 89-104.

As an approach toward understanding the mechanisms by which cadmium (Cd) exerts its teratogenic effects, the expression and metal regulation of the metallothionein (MT) genes in midgestation mouse embryos were studied by Northern blot and in situ hybridization. Maternal injection of a teratogenic dosage of Cd (50 mumol Cd/kg body wt) did not induce MT mRNA in day 10 (D10) CD-1 mouse embryos, whereas zinc (Zn) (50 mumol/kg was an effective inducer. In contrast, Cd was about 10-fold more potent than Zn at rapidly inducing MT mRNA in D10 embryos incubated in vitro in medium containing micromolar concentrations of these metals. This suggests that following maternal injection, Cd but not Zn is prevented from reaching the D10 embryo and establishes that the embryonic MT genes are not refractory to metal induction, which might have explained the sensitivity of the embryo to Cd. MT mRNA was detected at high levels only in the extraembryonic membranes of D9 embryos exposed to Cd in vivo. On days 9 and 10, no embryonic cell types contained detectable levels of MT mRNA. This mRNA was detected first at low levels in hepatocytes on D11, soon after formation of liver and these levels increased dramatically by D12. Therefore, Cd teratogenicity was not associated with high levels of cell type-specific expression of the MT genes in Cd-sensitive regions of the embryo (neural tube, limb bud), that might have served to target Cd to these cells. Taken together, the results of this study suggest that Cd teratogenicity reflects damage to maternal or extraembryonic tissues. However, the results cannot exclude the possibility that certain cells in the embryo are exceptionally sensitive to low levels of Cd.

De, S. K., et al. (1990). "Endotoxin induction of murine metallothionein gene expression." J Biol Chem 265(25): 15267-15274.

Bacterial endotoxin-lipopolysaccharide (LPS) rapidly induced hepatic metallothionein (MT) mRNA levels in the LPS-sensitive CD-1 strain of mice. This LPS effect was severely attenuated in the LPS-resistant C3H/HeJ strain of mice, but could be mimicked by injection of human recombinant interleukin-1 alpha (IL-1 alpha) or human recombinant tumor necrosis

factor (TNF-alpha). In the CD-1 strain, LPS induction of MT gene expression occurred in each of 10 organs examined (liver, kidney, pancreas, intestine, lung, heart, brain, ovary, uterus, and spleen). Solution hybridization with probes specific for MT-I or MT-II mRNA established that these genes were co-induced in each of the organs and that the liver and kidney contained the highest absolute levels of these mRNAs, whereas in the intestine and spleen they were 10-20-fold lower. LPS and cytokine induction of hepatic MT gene expression occurred in hypophysectomized mice, which suggests a lack of significant involvement of glucocorticoids. Several recombinant cytokines (TNF-alpha, IL-1 alpha, IL-1 beta, IL-6, interferon-gamma (IFN-gamma), as well as poly(rI.rC) were effective inducers of hepatic MT-I and MT-II genes. As an attempt to determine which of these cytokines may mediate LPS effects on MT gene expression in vivo, CD-1 mice were injected with LPS or various cytokines, and RNA from liver, ovary, and uterus was extracted at various times postinjection and analyzed by Northern blotting using probes specific for IL-1 alpha, IL-1 beta, TNF-alpha, IL-6, and MT mRNA. In each organ examined, LPS, IL-1 alpha, or IL-1 beta injection caused a rapid, coordinate, transient increase in the levels of each of the cytokine mRNAs which peaked by 1 h and declined to low levels by 4 h. In contrast, levels of MT mRNA did not reach a peak until 4-6 h postinjection. TNF-alpha had minimal effects on expression of cytokine and MT genes in organs other than liver. IL-6 had no effect on hepatic cytokine mRNA levels, and induced MT mRNA only in the liver which suggests a direct effect of IL-6 on hepatic MT gene expression. These data suggest that the acute effects of LPS on MT gene expression may include complex paracrine interactions between a variety of cytokines and the cells expressing MT genes in each organ, and tissue-specific cytokine effects on the MT genes.

De, S. K., et al. (1989). "Cell-specific metallothionein gene expression in mouse decidua and placentae." Development 107(3): 611-621.

Oligodeoxyribonucleotide excess solution hybridization, Northern blot and in situ hybridization were used to analyze metallothionein gene expression in mouse decidua and placentae during gestation. Metallothionein (MT) -I and -II mRNA levels were constitutively elevated, 11- and 13-fold, respectively, relative to the adult liver, in the deciduum (D8), and decreased coordinately about 6-fold during the period of development when the deciduum is replaced by the developing placenta (D10-16). Coincident with this decline, levels of MT mRNA increased dramatically in the visceral yolk sac endoderm. In situ hybridization established that MT-I mRNA was present at low levels in the uterine luminal epithelium (D4), but was elevated at the site of embryo implantation exclusively in the primary decidual zone by D5, and then in the secondary decidual zone (D6-8). Although low levels of MT mRNA were detected in total placental RNA, in situ hybridization revealed constitutively high levels in the outer placental spongiotrophoblasts. Analysis of pulse-labeled proteins from decidua and placentae established that these tissues are active in the synthesis of MT. The constitutively high levels of MT mRNA in decidua were only slightly elevated following injection of cadmium (Cd) and/or zinc (Zn), whereas in placentae they increased several-fold. MT mRNA levels were equally high in decidua and experimentally induced deciduomata (D8) which establishes that decidual MT gene expression is not dependent on the presence of the embryo or some embryo-derived factor. Although the functional role of MT during development is speculative, these results establish the concept that, from the time of implantation to late in gestation, the mouse embryo is surrounded by cells, interposed between the maternal and embryonic environments, which actively express the MT genes. This suggests that MT plays an important role in the establishment and maintenance of normal pregnancy.

del Carmen, E. M., et al. (2002). "Cadmium induces alpha(1)collagen (I) and metallothionein II gene and alters the antioxidant system in rat hepatic stellate cells." Toxicology 170(1-2): 63-73.

The mechanism of cadmium-mediated hepatotoxicity has been the subject of numerous investigations, principally in hepatocytes. Although, some uncertainties persist, sufficient evidence has emerged to provide a reasonable account of the toxic process in parenchymal cells. However, there is no information about the effect of cadmium in other hepatic cell types, such as stellate cells (fat storing cells, Ito cells, perisinusoidal cells, parasinusoidal cells, lipocytes). Hepatic stellate cells (HSC) express a quiescent phenotype in a healthy liver and acquire an activated phenotype in liver injury. These cells play an important role in the fibrogenic process. The objective of this study was to investigate the effect of a 24 h treatment of low Cd concentrations in glutathione content, lipid peroxidation damage, cytosolic free Ca, antioxidant enzyme activities: glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase along with the capacity of this heavy metal to induce metallothionein II and alpha(1)collagen (I) in an hepatic stellate cell line (CFSC-2G). Cd-treated cells increased lipid peroxidation and the content of cytosolic free calcium, decreased glutathione content and superoxide dismutase, glutathione peroxidase and catalase activity. Cd was able to induce the expression of the metallothionein II and alpha(1)collagen (I) gene, that was not described in this cell type. Cadmium may act as a pro-fibrogenic agent in the liver probably by inducing oxidative damage by enhancing lipid

peroxidation and altering the antioxidant system of the cells. Although, the exact role metallothionein induction plays in this process is unknown, it probably, provides a cytosolic pool of potential binding sites to sequester ionic Cd, thereby decreasing its toxicity.

Dixit, A., et al. (1989). "A cis-acting sequence within the rat ribosomal DNA enhancer region can modulate RNA polymerase II-directed transcription of the metallothionein I gene in vitro." DNA 8(5): 311-320.

Plasmids were constructed by inserting a 557-bp or 174-bp spacer fragment of rat ribosomal (r)DNA containing an enhancer element(s) at -148 bp upstream from a cloned mouse metallothionein gene (pMT-I). Transcription of these plasmids in a fractionated nuclear extract from a rat hepatoma resulted in 5 to 20-fold stimulation of MT-I gene transcription. This enhancement occurred independent of orientation of the enhancer or its distance from the metallothionein gene promoter or in the presence of the MT-I gene enhancer, and was sensitive to low levels of alpha-amanitin. Stimulation of MT-I gene transcription under the direction of the rDNA spacer element also occurred in HeLa nuclear extract, albeit to a smaller extent. Prior incubation of the nuclear extract with the 557-bp or 174-bp fragment resulted in as much as 5- to 10-fold stimulation of MT-I gene transcription. No significant effect on MT-I gene transcription was observed following preincubation with other DNAs. Preincubation of the extract with three subfragments of the 174-bp spacer inhibited MT-I gene transcription, which suggests that the majority of the 174-bp domain is required for binding to the negative regulatory factor(s) for MT-I gene transcription and that the subfragments can only interact with the positive core promoter-binding factor. The 37-bp subfragment, which has been shown to interact with a positive rDNA trans-acting factor, could also interact with a positive polymerase II (pol II) trans-acting factor. These studies have demonstrated that the 174-bp rat rDNA spacer element containing the pol I enhancer can also modulate pol II-directed transcription.

Do, M. S., et al. (2002). "Metallothionein gene expression in human adipose tissue from lean and obese subjects." Horm Metab Res 34(6): 348-351.

Expression of the gene encoding metallothionein, a low molecular-weight cysteine-rich, stress-response and metal-binding protein was examined in human adipose tissue. The mRNA for MT-2A, a major metallothionein isoform in humans, was detected in subcutaneous fat using a specific antisense oligonucleotide probe. The level of MT-2A mRNA was significantly higher in a group of obese subjects than in a lean group, paralleling a similar increase in ob mRNA. A two-week period on a diet of 800 calories/day did not lead to any significant change in MT-2 mRNA levels. Separation of mature adipocytes from the cells of the stromal vascular fraction indicated that in human adipose tissue the metallothionein (MT-2A) gene is expressed both in adipocytes and in other cells of the tissue.

Dondero, F., et al. (2004). "Biochemical characterization and quantitative gene expression analysis of the multi-stress inducible metallothionein from Tetrahymena thermophila." Protist 155(2): 157-168.

A cadmium-binding protein with biochemical features of a metallothionein (MT) has been isolated and purified to homogeneity from the ciliate Tetrahymena thermophila. N-terminal sequencing revealed the posttranslational cleavage of the first two amino acids and, in general, a high degree of identity with known MTs from other ciliates. Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) analysis of the apothionein revealed a molecular mass (16,763 Da) higher to those of mammals and of other protozoa. Finally, quantitative real-time PCR has been used to investigate the susceptibility of this ciliate MT to gene activation in response to heavy metals and to other stressors. Our data indicate that while zinc is not effective at all and cadmium is the best inducer, other stress factors, such as mercury, copper, heat and hydrogen peroxide, also activated gene transcription. As in vertebrate cells, interleukin-6 (IL-6) that stimulates ciliate growth, was able to enhance MT gene synthesis. This complex of data seems to indicate a general role of this protein in stress response.

Dong, C. J., et al. (2010). "Characterization of a novel rice metallothionein gene promoter: its tissue specificity and heavy metal responsiveness." J Integr Plant Biol 52(10): 914-924.

The rice (Oryza sativa L.) metallothionein gene OsMT-I-4b has previously been identified as a type I MT gene. To elucidate the regulatory mechanism involved in its tissue specificity and abiotic induction, we isolated a 1 730 bp fragment of the OsMT-I-4b promoter region. Histochemical beta-glucuronidase (GUS) staining indicated a precise spacial and temporal expression pattern in transgenic Arabidopsis. Higher GUS activity was detected in the roots and the buds of flower stigmas, and relatively lower GUS staining in the shoots was restricted to the trichomes and hydathodes of leaves. No activity was observed in the stems and seeds. Additionally, in the root of transgenic plants, the promoter activity was highly upregulated by various environmental signals, such as abscisic acid, drought, dark, and heavy metals including Cu(2)(+) , Zn(2)(+) , Pb(2)(+) and Al(3)(+) . Slight induction was observed in transgenic seedlings under salinity stress, or when treated with Co(2)(+) and Cd(2)(+) . Promoter analysis of 5'-deletions revealed that the region -583/-1 was sufficient to drive strong GUS expression in the

roots but not in the shoots. Furthermore, deletion analysis indicated important promoter regions containing different metal-responsive cis-elements that were responsible for responding to different heavy metals. Collectively, these findings provided important insight into the transcriptional regulation mechanisms of the OsMT-I-4b promoter, and the results also gave us some implications for the potential application of this promoter in plant genetic engineering.

Drucker, D. J., et al. (1986). "Cell-specific post-translational processing of preproglucagon expressed from a metallothionein-glucagon fusion gene." J Biol Chem 261(21): 9637-9643.

Glucagon is a peptide hormone of 29 amino acids encoded by a preprohormone which contains in tandem the sequences of glucagon and two additional glucagon-like peptides (GLPs) structurally related to glucagon and separated by intervening peptides. Glucagon arises by cleavage from the prohormone within the A cells of the pancreatic islets but in the intestine remains as part of a partially processed precursor (glicentin). To determine whether additional glucagon-like peptides are processed from preproglucagon and to analyze for potential cellular specificity in the processing of preproglucagon, we introduced and expressed a metallothionein-glucagon fusion gene in a fibroblast and two endocrine (pituitary and pancreatic islet) cell lines. Chromatographic analyses of cell extracts utilizing specific radioimmunoassays to chemically synthesized peptides demonstrate the liberation of intact glucagon, glicentin, GLP-I(1-37), GLP-I(7-37), GLP-II, and an intervening peptide amidated at its carboxyl terminus. The peptides were present in distinct yet different patterns in the two endocrine but not the fibroblast cell lines. The cell-specific liberation of the glucagon-like and intervening peptides suggests their potential as new bioactive peptides. The cellular specificity in the processing of preproglucagon indicates that the genetic determinants of the processing activity are complex and are expressed in a cell-specific manner.

Durliat, M., et al. (1999). "Expression of the Xenopus laevis metallothionein gene during ontogeny." Int J Dev Biol 43(6): 575-578.

Expression of the Xenopus laevis metallothionein (MT) gene was studied by in situ hybridization throughout development. MT mRNA was detected from the tailbud stage onwards. MT expression was observed in bucco-pharyngeal epithelium, pronephros and liver anlagen, aswell as in lens and periventricular areas of the encephalon. MT transcripts, in both larvae and adults, were detected in diverse regions of the central nervous system and in differentiating tissues implicated in detoxification processes: liver hepatocytes, small intestine epithelia and kidney tubules. These data are discussed in the context of MT functions and support a physiological role for MT in growth processes.

Durnam, D. M. and R. D. Palmiter (1981). "Transcriptional regulation of the mouse metallothionein-I gene by heavy metals." J Biol Chem 256(11): 5712-5716.

Administration of Cd, Zn, Cu, or Hg increases the rate of transcription from the metallothionein-I gene in mouse liver and kidney. Maximal transcription rates occur 1 h after Cd administration in both tissues. Metallothionein-I mRNA levels, measured by cDNA hybridization, and metallothionein protein synthesis, measured by [35S]cysteine incorporation, increase simultaneously, reaching maximal levels about 4 h after Cd administration. Cd also induces metallothionein-I mRNA in all other tissues examined (spleen, heart, skeletal muscle, brain, and intestine) except testes. Comparison of the inductions by Cd and Hg shows that the kinetics of metallothionein-I mRNA accumulation as well as the stability of the resultant metallothioneins differ.

Durnam, D. M., et al. (1980). "Isolation and characterization of the mouse metallothionein-I gene." Proc Natl Acad Sci U S A 77(11): 6511-6515.

Double-stranded cDNA was synthesized from a mouse liver mRNA fraction enriched for metallothionein mRNA activity, ligated to restriction site linkers, inserted into pBR322, and used to transform Escherichia coli chi 1776. The sequence of the largest plasmid containing DNA that hybridized to metallothionein mRNA was determined and shown to contain a 380-base-pair insert that includes the entire coding region and 3' untranslated region of metallothionein-I. The metallothionein-I insert was nick-translated and used to screen both a mouse myeloma and a mouse embryo DNA library in bacteriophage lambda. A metallothionein-I genomic clone containing 13-15 kilobase pairs of mouse DNA was isolated from each library. Both contain a 3.8-kilobase-pair EcoRI fragment that hybridizes to the metallothionein-I probe. The location, size, and orientation of the metallothionein-I gene within the 3.8-kilobase-pair fragment were determined by heteroduplex and restriction mapping. The gene spans 1.1 kilobase pairs and contains at least two introns.

Egg, M., et al. (2009). "Structural and bioinformatic analysis of the Roman snail Cd-Metallothionein gene uncovers molecular adaptation towards plasticity in coping with multifarious environmental stress." Mol Ecol 18(11): 2426-2443.

Metallothioneins (MTs) are a family of multifunctional proteins involved, among others, in stress response. The Cadmium (Cd)-MT gene of the Roman snail (Helix pomatia), for example, encodes for a protein induced upon cadmium exposure. While our

previous studies have demonstrated that the expressed Cd-MT isoform of Roman snails assists detoxification of cadmium, the present work focuses on the potential plasticity of this gene in response to a variety of environmental stressors playing a crucial role in the specific ecological niche of H. pomatia. Our hypothesis is based on a bioinformatic approach involving gene sequencing, structural and in silico analysis of transcription factor binding sites (TFBs), and a comparison of these features with other MT genes. Our results show that the Roman snail's Cd-MT gene not only is the largest known MT gene, but also contains--apart from the regulatory promoter region--several intronic repeat cassettes of putative TFBs suggested to be involved in environmental stress response, immune competence, and regulation of gene expression. Moreover, intronic scaffold/matrix attachment regions (S/MARs) and stress-induced duplex destabilization sites confer a high potential for epigenetic gene regulation. This suggested regulatory plasticity is also supported by physiological data showing that Cd-MT in Roman snails can be induced differentially not only after cadmium exposure, but also in response to nonmetallic environmental stressors. It is concluded that structural analysis combined with bioinformatic screening may constitute valuable tools for predicting the potential for plasticity and niche-specific adaptation of stress-responsive genes in populations living under rapidly changing environmental conditions.

Emeny, R. T., et al. (2009). "Manipulations of metallothionein gene dose accelerate the response to Listeria monocytogenes." Chem Biol Interact 181(2): 243-253.

Metallothioneins (MTs) are cysteine-rich proteins that assist in cellular homeostasis and protect against oxidant injury. MTs can be induced by heavy metals and inflammatory mediators and function as free radical scavengers, reservoirs for essential heavy metals, and immunomodulators. In light of MTs' roles in responses to stress, we evaluated the in vivo effects of MT gene dose on the course of Listeria monocytogenes (LM) infection. LM burden was measured in livers and spleens, and flow cytometric assays were used to analyze splenocyte surface sulfhydryls, oxidative burst and apoptosis. Our results suggest that deviations from the normal complement of MT genes alter the course of LM infection. Compared to the wild-type C57BL/6J (B6-WT) strain, a congenic partner that carries a larger number of Mt1 genes (B6-MTTGN) and a congenic strain in which both Mt1 and Mt2 are disrupted (B6-MTKO) both showed lower bacterial burdens three days post-inoculation. This difference was prominent in the first 48h of infection, after which LM clearance occurred at comparable rates in all three strains. Lymphocytes from B6-MTKO mice exhibited increased cell death and increased levels of surface sulfhydryls compared to B6-WT and B6-MTTGN mice. Lymphocytes from B6-MTTGN mice had increased levels of intracellular oxidants compared to B6-WT and B6-MTKO mice. The oxidative burst by macrophages from infected B6-MTTGN and B6-MTKO mice was increased, suggesting one mechanism by which these strains might reduce the LM burden. These results indicate that MT gene dose dramatically influences host-defenses against LM infection.

Ercolani, L., et al. (1990). "Membrane localization of the pertussis toxin-sensitive G-protein subunits alpha i-2 and alpha i-3 and expression of a metallothionein-alpha i-2 fusion gene in LLC-PK1 cells." Proc Natl Acad Sci U S A 87(12): 4635-4639.

The renal epithelial cell line LLC-PK1 has topographically distinct regulatory roles for the alpha subunits of pertussis toxin-sensitive guanine nucleotide regulatory proteins (alpha i subunit); these include the inhibition of adenylyl cyclase at the basolateral membrane and the stimulation of Na+ channel activity at the apical membrane. We now report that LLC-PK1 cells contain two members of the alpha i protein family, alpha i-2 and alpha i-3, which have distinct cellular locations consistent with their diverse functional roles. By using specific alpha i antibodies and immunofluorescence, the alpha i-2 subunit was found to be localized to the basolateral membrane, whereas the alpha i-3 subunit was concentrated in the Golgi and was also detectable at low levels on apical membranes in some cells. Induction of a chimeric mouse metallothionein 1-rat or canine alpha i-2 gene stably transfected into the LLC-PK1 cells produced an increase in the content of the alpha i-2 subunit, which was targeted only to the basolateral membrane. These findings suggest that alpha i subunit specificity for effectors may be achieved in polarized renal epithelial cells by their geographic segregation to different cellular membranes. The LLC-PK1 cell stably transfected with the metallothionein-alpha i-2 fusion gene will provide a model for the study of guanine nucleotide regulatory protein function in epithelia.

Erickson, J. C., et al. (1997). "Disruption of the metallothionein-III gene in mice: analysis of brain zinc, behavior, and neuron vulnerability to metals, aging, and seizures." J Neurosci 17(4): 1271-1281.

Metallothionein-III (MT-III), a brain-specific member of the metallothionein family of metal-binding proteins, is abundant in glutamatergic neurons that release zinc from their synaptic terminals, such as hippocampal pyramidal neurons and dentate granule cells. MT-III may be an important regulator of zinc in the nervous system, and its absence has been implicated in the development of Alzheimer's disease. However, the roles of MT-III in brain physiology and pathophysiology have not been elucidated. Mice

lacking MT-III because of targeted gene inactivation were generated to evaluate the neurobiological significance of MT-III. MT-III-deficient mice had decreased concentrations of zinc in several brain regions, including hippocampus, but the pool of histochemically reactive zinc was not disturbed. Mutant mice exhibited normal spatial learning in the Morris water maze and were not sensitive to systemic zinc or cadmium exposure. No neuropathology or behavioral deficits were detected in 2-year-old MT-III-deficient mice, but the age-related increase in glial fibrillary acidic protein expression was more pronounced in mutant brain. MT-III-deficient mice were more susceptible to seizures induced by kainic acid and subsequently exhibited greater neuron injury in the CA3 field of hippocampus. Conversely, transgenic mice containing elevated levels of MT-III were more resistant to CA3 neuron injury induced by seizures. These observations suggest a potential role for MT-III in zinc regulation during neural stimulation.

Espinoza, H. M., et al. (2012). "Effect of cadmium on glutathione S-transferase and metallothionein gene expression in coho salmon liver, gill and olfactory tissues." Aquat Toxicol 110-111: 37-44.

The glutathione S-transferases (GSTs) are a multifunctional family of phase II enzymes that detoxify a variety of environmental chemicals, reactive intermediates, and secondary products of oxidative damage. GST mRNA expression and catalytic activity have been used as biomarkers of exposure to environmental chemicals. However, factors such as species differences in induction, partial analyses of multiple GST isoforms, and lack of understanding of fish GST gene regulation, have confounded the use of GSTs as markers of pollutant exposure. In the present study, we examined the effect of exposure to cadmium (Cd), a prototypical environmental contaminant and inducer of mammalian GST, on GST mRNA expression in coho salmon (Oncorhynchus kisutch) liver, gill, and olfactory tissues. GST expression data were compared to those for metallothionein (MT), a prototypical biomarker of metal exposure. Data mining of genomic databases led to the development of quantitative real-time PCR (qPCR) assays for salmon GST isoforms encompassing 9 subfamilies, including alpha, mu, pi, theta, omega, kappa, rho, zeta and microsomal GST. In vivo acute (8-48 h) exposures to low (3.7 ppb) and high (347 ppb) levels of Cd relevant to environmental scenarios elicited a variety of transient, albeit minor changes (<2.5-fold) in tissue GST profiles, including some reductions in GST mRNA expression. In general, olfactory GSTs were the earliest to respond to cadmium, whereas, more pronounced effects in olfactory and gill GST expression were observed at 48 h relative to earlier time points. Although evaluation of GSTs reflected a cadmium-associated oxidative stress response, there was no clear GST isoform in any tissue that could serve as a reliable biomarker of acute cadmium exposure. By contrast, metallothionein (MT) mRNA was consistently and markedly induced in all three tissues by cadmium, and among the tissues examined, olfactory MT was the most sensitive marker of cadmium exposures. In summary, coho salmon exhibit a complex GST tissue profile consisting of at least 9 isoforms, all of which are present in the peripheral olfactory system. Short-term exposure to environmental levels of Cd causes transient changes in salmon GST consistent with oxidative stress, and in some cases, includes a loss of GST. In a biomarker context, however, monitoring of tissue MT mRNA expression, especially in the peripheral olfactory system, may be of greater utility for assessing short-term environmental exposures to cadmium.

Evans, I. M., et al. (1990). "A gene from pea (Pisum sativum L.) with homology to metallothionein genes." FEBS Lett 262(1): 29-32.

While searching for 'organ-specific' genes in pea (Pisum sativum L.) we have isolated a gene (designated PsMTA) which has an ORF encoding a predicted protein with some similarity to metallothioneins (MTs). The PsMTA transcript is abundant in roots which have not been exposed to elevated concentrations of trace metals.

Evans, K. M., et al. (1992). "Expression of the pea metallothionein-like gene PsMTA in Escherichia coli and Arabidopsis thaliana and analysis of trace metal ion accumulation: implications for PsMTA function." Plant Mol Biol 20(6): 1019-1028.

The PsMTA gene from pea (Pisum sativum) shares similarity with metallothionein (MT) genes and related sequences have also been isolated from a number of other higher-plant species. The proteins encoded by these genes have not yet been purified from plants and their functions remain unclear although, by analogy to MT, roles in the metabolism and detoxification of metal ions have been proposed. By contrast, correlation between transcript abundance and Fe availability has led to an alternative proposal that these genes are involved in mechanisms of Fe efficiency. Phenotypic effects of constitutive PsMTA expression were examined in Escherichia coli and Arabidopsis thaliana. Copper accumulation by E. coli cells expressing recombinant PsMTA protein was approximately 8-fold greater than in control cells. No significant effects on the accumulation of Zn or Cd were detected. In segregating A. thaliana progeny, derived from a transgenic F1 parent containing the PsMTA gene under the control of a CaMV 35S promoter, 75% of individuals accumulated more Cu (several-fold in some plants) than untransformed, control plants. These data suggest that PsMTA protein

binds Cu in planta and that uncoupled (constitutive) expression of the PsMTA gene causes enhanced Cu accumulation. Roots of P. sativum plants grown under conditions of low Fe availability showed elevated activity of root surface Fe(III) reductase and accumulated more Cu than roots of plants grown in an Fe-supplemented solution. Changes in the expression of MT-like genes, coincident with changes in Fe availability, are consistent with a role in Cu homoeostasis.

Faraonio, R., et al. (2000). "Characterization of cis-acting elements in the promoter of the mouse metallothionein-3 gene. Activation of gene expression during neuronal differentiation of P19 embryonal carcinoma cells." Eur J Biochem 267(6): 1743-1753.

The metallothionein (MT)3 gene is expressed predominantly in the brain and the organs of the reproductive system, and fails to respond to metal ions in vivo. A CTG repeat was proposed to function as a potential repressor element in nonpermissive cells, and a sequence similar to the JC virus silencer element was found to function as a negative element in permissive primary astrocytes. The objective of this study was to characterize further the mechanisms governing cell-type specific MT-3 gene transcription. We searched for a suitable cell line expressing the MT-3 gene to be used for determination of MT-3 promoter tissue specificity, and showed that MT-3 expression is activated during neuroectodermal differentiation of P19 cells induced by retinoic acid to levels similar to those found in whole brain. Deletion of the CTG repeat or of the JC virus silencer did not promote MT-3 promoter activity in nonpermissive cells, or enhance expression in permissive cells. We identified MT-3 promoter sequences interacting with liver and brain nuclear proteins, as assayed by DNase I footprinting analyses and electrophoretic mobility shift assay, and assessed the role of these sequences in the regulation of MT-3 expression by cotransfection experiments. We generated stable transfectants in permissive C6 and nonpermissive NIH-3T3 cells, and analysed the methylation status of the MT-3 gene. These studies show that regulation of tissue-specific MT-3 gene expression does not appear to involve a repressor, and suggest that other mechanisms such as chromatin organization and epigenetic modifications could account for the absence of MT-3 gene transcription in nonpermissive cells.

Felix-Portillo, M., et al. (2014). "The metallothionein gene from the white shrimp Litopenaeus vannamei: characterization and expression in response to hypoxia." Mar Environ Res 101: 91-100.

Aquatic animals encounter variation in oxygen tension that leads to the accumulation of reactive oxygen species (ROS) that can harm the organisms. Under these circumstances some organisms have evolved to tolerate hypoxia. In mammals, metallothioneins (MTs) protect against hypoxia-generated ROS. Here we report the MT gene from the shrimp Litopenaeus vannamei (LvMT). LvMT is differentially expressed in hemocytes, intestine, gills, pleopods, heart, hepatopancreas and muscle, with the highest levels in hepatopancreas and heart. LvMT mRNA increases during hypoxia in hepatopancreas and gills after 3 h at 1.5 mg L(-1) dissolved oxygen (DO). This gene structure resembles the homologs from invertebrates and vertebrates possessing three exons, two introns and response elements for metal response transcription factor 1 (MTF-1), hypoxia-inducible factor 1 (HIF-1) and p53 in the promoter region. During hypoxia, HIF-1/MTF-1 might participate inducing MT to contribute towards the tolerance to ROS toxicity. MT importance in aquatic organisms may include also ROS-detoxifying processes.

Fernando, L. P. and G. K. Andrews (1989). "Cloning and expression of an avian metallothionein-encoding gene." Gene 81(1): 177-183.

The chicken metallothionein gene (cMT), isolated from a chicken genomic DNA phage lambda library, was found to be approximately 1.5 kb in length and to consist of three exons, separated by two intervening sequences. The number and placement of the introns in the cMT gene is precisely the same as that in the mammalian metallothionein-coding genes. S1 nuclease mapping indicated a prominent transcription start point (tsp) 62 bp 5' to the translation start codon. The promoter region analyzed (623 bp) contained three regions of homology (at -47, -488, and -577 bp relative to the tsp) with the metal regulatory element (MRE) consensus sequence, and three potential Sp1 binding sites. Two of the putative MREs (-47, -577) were 12 to 14-bp palindromes, which suggests that they are binding sites for trans-acting proteins. The intact cMT gene was functional in mammalian cells, and the cMT promoter could confer metal responsiveness on the firefly luciferase cDNA (Luc) in transient expression assays. Deletion mutagenesis established that 107 bp of 5'-flanking sequence, containing the proximal MRE and a putative Sp1-binding site, were sufficient for transient expression and metal induction of the cMT promoter-Luc fusion gene.

Fisker, K. V., et al. (2013). "Variation in metallothionein gene expression is associated with adaptation to copper in the earthworm Dendrobaena octaedra." Comp Biochem Physiol C Toxicol Pharmacol 157(2): 220-226.

Evolution of resistance to heavy metals has been reported for several populations of soil living organisms occurring at metal contaminated sites. Such genetically based and heritable resistance contribute to

the persistence of populations in contaminated areas. Here we report on molecular responses to experimental copper in populations of the earthworm, Dendrobaena octaedra, originating from copper contaminated soil near Gusum (Sweden) where heavy metal pollution has been present for several decades. We studied gene expression of six genes potentially involved in resistance to copper toxicity using F2-generations of D. octaedra populations, originating from reference sites and contaminated (High, Medium and Low) sites around Gusum. The main result was different expression patterns of genes encoding for two different isoforms (mt1 and mt2) of metallothionein proteins during experimental exposure to copper contaminated soil. Expression of mt1 showed a fast and significant upregulation in the High population and a slower, albeit significant, upregulation in Medium and Low populations. However, in the three reference populations no upregulation were seen. In comparison, a fast upregulation was also seen for the High population in the isoform mt2, whereas, gene expression of all other populations, including reference populations, showed slower upregulation in response to experimental copper. The results indicate that copper resistance in D. octaedra from contaminated areas is related to an increased expression of metallothioneins.

Fisker, K. V., et al. (2016). "Freezing of body fluids induces metallothionein gene expression in earthworms (Dendrobaena octaedra)." Comp Biochem Physiol C Toxicol Pharmacol 179: 44-48.

The molecular mechanisms activated by environmental contaminants and natural stressors such as freezing need to be investigated in order to better understand the mechanisms of interaction and potential effects that combined stressors may have on organisms. Using the freeze-tolerant earthworm Dendrobaena octaedra as model species, we exposed worms to freezing and exposure to sublethal copper in a factorial design and investigated the transcription of candidate genes for metal and cold stress. We hypothesised that both freezing and copper would induce transcription of genes coding for heat shock proteins (hsp10 and hsp70), metallothioneins (mt1 and mt2), and glutathione-S-transferase (gst), and that the combined effects of these two stressors would be additive. The gene transcripts hsp10, hsp70, and gst were significantly upregulated by freezing, but only hsp10 was upregulated by copper. We found that copper at the time of sampling had no effect on transcription of two metallothionein genes whereas transcription was strongly upregulated by freezing. Moreover, there was a significant interaction causing more than additive transcription rates of mt1 in the copper/freezing treatment suggesting that freeze-induced cellular dehydration increases the concentration of free copper ions in the cytosol. This metallothionein response to freezing is likely adaptive and possibly provides protection against freeze-induced elevated metal concentrations in the cytosol and excess ROS levels due to hypoxia during freezing.

Foley, R. C. and K. B. Singh (1994). "Isolation of a Vicia faba metallothionein-like gene: expression in foliar trichomes." Plant Mol Biol 26(1): 435-444.

Animal metallothioneins (MTs) are cysteine-rich, low-molecular-weight proteins that bind to heavy metals and are believed to play a role in their metabolism and detoxification. Genes encoding MT-like proteins have been isolated in a number of plants although their function remains to be elucidated. We describe the isolation and characterization of a bean cDNA encoding an MT-like protein. The bean gene, called MT, was isolated as a result of a differential screen for genes that are expressed in leaves but not in the most common cell type, the mesophyll cell. MT contained two regions with abundant cysteines and sequence comparison found that MT had greatest homology to MT-like subtype 2 from other plant species. Northern blot analysis demonstrated that MT was expressed in the left, stem and flower, at very low levels in roots and was not detectable in mesophyll protoplasts. MT transcript levels were not significantly affected by treatment with Cu, Zn or Cd. In the left, in situ hybridization studies demonstrated striking cell specificity with MT expression confined predominantly to trichomes. Possible explanations for the pronounced expression of MT in leaf trichomes are discussed.

Fordham-Skelton, A. P., et al. (1997). "GUS expression in Arabidopsis directed by 5' regions of the pea metallothionein-like gene PsMTA." Plant Mol Biol 34(4): 659-668.

Upstream sequences (including the first seven codons) of a metallothionein (MT)-like gene from pea, PsMTA, were fused to GUS and introduced into Arabidopsis. High-level GUS expression was detected in the roots of plants grown on MS medium, except in regions proximal to the root apex. There was precise delineation of the root-shoot boundary. In soil-grown plants there was low GUS expression and this was absent from the more mature regions of the roots. In the aerial tissues of soil-grown plants, GUS expression was restricted to hydathodes, stipules, expanding cotyledons and the following senescent tissues: leaves, cotyledons, petals, sepals, filaments, stigmas, nectaries and siliques. A 298 bp region was shown to be required for GUS expression in roots but not for expression in vegetative aerial tissues of plants grown on MS medium. This region contains predicted ethylene-responsive elements (EREs) but similar patterns of GUS expression were detected in etr1 seedlings. GUS expression was significantly higher in roots exposed to 500 nM copper, but this increase was small in proportion to expression in roots exposed to 50 nM copper.

Formigari, A., et al. (2010). "Functional characterization of the 5'-upstream region of MTT5 metallothionein gene from Tetrahymena thermophila." Protist 161(1): 71-77.

Metallothioneins are ubiquitous small, cysteine-rich, metal-binding proteins that play important roles in intracellular metal homeostasis and detoxification. Very few data are available on the promoter region and the mechanism of metallothionein transcription in Protozoa. In this study, we focused on Tetrahymena thermophila MTT5 5'-flanking region. To define the sequence elements underlying the metal-responsiveness of this promoter, we constructed a series of deletions and mutations starting with a 1777 bp fragment immediately upstream of the start codon of MTT5. As a reporter gene we used the previously tested IAG52B surface antigen from the protozoan fish parasite Ichthyophthirius multifiliis. The results suggest that a region spanning between -300 bp and -274 bp, dubbed Tetrahymena thermophila Cadmium-Response-Element (TtCdRE), is necessary to elicit high-level expression of the transgene following induction with cadmium. This is the first demonstration by in vivo analyses of a regulatory element essential for Cd-mediated control of protozoan metallothionein gene expression, where the sequence GATA appears to be involved.

Foster, R., et al. (1988). "Structure and expression of the human metallothionein-IG gene. Differential promoter activity of two linked metallothionein-I genes in response to heavy metals." J Biol Chem 263(23): 11528-11535.

The human metallothionein (MT)-IG gene (hMT-IG) is tandemly linked in a head-to-head fashion with the hMT-IF gene. The hMT-IG gene encodes a MT-I polypeptide and has a tripartite structure. The 5'-flanking region of the hMT-IG gene has a TATAA box, four GC motifs, and at least four metal responsive elements. The 3'-untranslated region has a variation of the polyadenylation signal, AATTAA, and the 3'-flanking region a YGTGTTYY RNA processing signal. This gene is expressed in hepatoma-derived cell lines (Hep G2 and Hep3B2) in response to the heavy metals (cadmium, copper, and zinc) but not to the glucocorticoid analogue dexamethasone. In contrast, the lymphoblastoid cell line (Wi-L2) does not express the hMT-IG gene. These results suggest that the hMT-IG gene is regulated differentially and in a cell type-specific manner. Transient expression studies of the chloramphenicol acetyltransferase (CAT) gene under the transcriptional control of either the hMT-IG or hMT-IF promoter in Hep G2 cells has demonstrated that both promoters contain all the necessary cis-acting elements to elicit a similar pattern of heavy metal inducibility. However, the hMT-IG promoter in all instances is five times more active than the hMT-IF promoter. The differences in promoter activity of these genes could possibly be due to inherent differences in their basal level regulatory sequences. The expression of MT-IGcat in transfected Wi-L2 cells demonstrates that the hMT-IG promoter is not cell type-specific.

Foster, R., et al. (1989). "Calcium phosphate-mediated transfection alters metallothionein gene expression in response to Cd2+ and Zn2+." Mol Cell Biol 9(9): 4105-4108.

The level of expression of a transfected metallothionein (MT)-IGcat fusion gene in response to cadmium differed from that of the endogenous MT-IG gene. Atomic absorption analysis indicated that the total cellular content of cadmium and zinc increased upon calcium phosphate-mediated transfection. Thus, changes in the influx/efflux of metals may regulate the level of MT gene expression.

Franchi, N., et al. (2011). "CiMT-1, an unusual chordate metallothionein gene in Ciona intestinalis genome: structure and expression studies." J Exp Zool A Ecol Genet Physiol 315A(2): 90-100.

The present article reports on the characterization of the urochordate metallothionein (MT) gene, CiMT-1, from the solitary ascidian Ciona intestinalis. The predicted protein is shorter than other known deuterostome MTs, having only 39 amino acids. The gene has the same tripartite structure as vertebrate MTs, with some features resembling those of echinoderm MTs. The promoter region shows the canonical cis-acting elements recognized by transcription factors that respond to metal, ROS, and cytokines. Unusual sequences, described in fish and echinoderms, are also present. In situ hybridization suggests that only a population of hemocytes involved in immune responses, i.e. granular amebocytes, express CiMT-1 mRNA. These observations support the idea that urochordates perform detoxification through hemocytes, and that MTs may play important roles in inflammatory humoral responses in tunicates. The reported data offer new clues for better understanding the evolution of these multivalent proteins from non-vertebrate to vertebrate chordates and reinforce their functions in detoxification and immunity.

Fu, C. and W. Miao (2006). "Cloning and characterization of a new multi-stress inducible metallothionein gene in Tetrahymena pyriformis." Protist 157(2): 193-203.

A new multi-stress-inducible metallothionein (MT) gene isoform has been cloned and characterized from the ciliate Tetrahymena pyriformis. Both the 5'- and 3'-UT regions of the Tp-MT2 gene are very different from the previously reported Tp-MT1 isoform in this organism and from other described MT genes in Tetrahymena pigmentosa and Tetrahymena thermophila. The putative protein sequence of Tp-MT2 contains cysteine clusters with characteristics of the typical

Tetrahymena Cd-inducible MT genes. However, the sequence has a special feature of four intragenic tandem repeats within its first half, with a conserved structural pattern x(5/8)CCCx(6)CCx(6)CxCxNCxCCK. To investigate the transcriptional activities of both Tp-MT2 and Tp-MT1 genes toward heavy metals (Cd, Hg, Cu, Zn) and H(2)O(2), the mRNA levels of these two isoforms were evaluated by means of real-time quantitative PCR. Results showed that Tp-MT2 had a higher basal expression level than Tp-MT1 and both genes were induced by Cd, Hg, Cu, and Zn ions after short exposure (1h), although to different extents. Cd was the most effective metal inducer of both two isoforms, but the relative expression level of Tp-MT2 was much lower than that of Tp-MT1. Different expression patterns were also shown between the two genes when treated with Cd over a period of 24h. We suggest that TpMT-1 plays the role of a multi-inducible stress gene, while TpMT-2 may have a more specific function in basal metal homeostasis although it may have undergone a functional differentiation process. The putative functional significance and evolutionary mode of the TpMT-2 isoform are discussed.

Fukuzawa, H., et al. (2004). "The rice metallothionein gene promoter does not direct foreign gene expression in seed endosperm." Plant Cell Rep 23(4): 231-235.

We generated transgenic tobacco and rice plants harboring a chimeric gene consisting of the 5'-upstream sequence of the rice metallothionein gene (ricMT) fused to the beta-glucuronidase (GUS) gene. The activity and tissue-specific expression of the ricMT promoter were demonstrated in these transgenic plants. In the transgenic rice plants, despite substantial levels of GUS activity in the shoot and root, almost no GUS signal was detected in the endosperm. Thus, the ricMT promoter could be useful in avoiding accumulation of undesired proteins in the seed endosperm.

Furst, P., et al. (1988). "Copper activates metallothionein gene transcription by altering the conformation of a specific DNA binding protein." Cell 55(4): 705-717.

Copper homeostasis in yeast involves a copper binding protein, metallothionein, and a trans-acting regulatory protein that activates transcription of the metallothionein gene in response to copper ions. We show that the regulatory protein specifically binds to the metallothionein gene control sequences in the presence, but not in the absence, of copper. Both the DNA binding and metalloregulatory functions of the transacting factor are contained within its aminoterminal domain, and partial proteolysis experiments show that copper activates this domain by causing a major switch in its conformation. Silver also activates the DNA binding domain in vitro and induces metallothionein gene transcription in vivo. We propose a novel copper cluster model for the DNA binding domain based on its surprising structural similarities to metallothionein itself.

Ganasyam, S. R., et al. (2012). "Association of Estrogen Receptor-alpha Gene & Metallothionein-1 Gene Polymorphisms in Type 2 Diabetic Women of Andhra Pradesh." Indian J Clin Biochem 27(1): 69-73.

Type 2 diabetes mellitus (DM) is a multifactorial disease where both genetic and environmental factors contribute to its pathogenesis. Estrogen plays an important role in type 2 DM pathogenesis. A number of polymorphisms have been reported in the estrogen receptor (ESR1), including the XbaI and PvuII restriction enzyme polymorphisms of ESR1,which may be involved in disease pathogenesis. Metallothioneins (MT) act as potent antioxidants against various oxidative damages. Very few studies have indicated the association between Estrogen Receptor-alpha, MT1 gene polymorphisms with type2 DM. A total of 100 type 2 diabetic women and 100 age, sex matched controls were recruited. Using the PCR based RFLP method, the PvuII and XbaI polymorphisms of ESR1 and in MT1A (rs8052394 and rs11076161) gene polymorphisms were analysed. The genotype distribution and frequency of mutated allele showed no significant differences between diabetic and non-diabetic groups in PvuII (chi2 = 2.443; P = 0.1181) or XbaI (chi2 = 1.789; P = 0.1812) and rs8052394 (chi2 = 1.154; P = 0.2840) or rs11076161 (chi2 = 0.4141; P = 0.5199), polymorphisms. This is the first Indian study to conclude that ESR1 and MT1 gene polymorphisms are not associated with increased susceptibility to type 2 diabetes in Indian women.

Ganguly, S., et al. (1996). "Human metallothionein gene expression determined by quantitative reverse transcription-polymerase chain reaction as a biomarker of cadmium exposure." Cancer Epidemiol Biomarkers Prev 5(4): 297-301.

Expression of the metallothionein (MT) gene in frozen human lymphocytes has been developed as a new molecular biomarker of heavy metal exposure. Workers at a Polish battery factory with high exposure to cadmium were monitored for airborne exposure and blood cadmium levels. A novel quantitative reverse transcription-PCR (RT-PCR) technique, making use of a homologous internal standard, was used to assess the level of MT-specific mRNA in frozen stored aliquots of blood samples taken from exposed and control workers. Results from this assay showed a statistically significant 2.5-fold increase in MT mRNA in exposed compared to control workers. The RT-PCR results also showed significant correlation with airborne cadmium, as registered on personal monitors and with blood cadmium levels. The results suggest that gene induction measured by quantitative RT-PCR is a

promising approach for application as a biomarker of biologically effective dose in small samples of frozen tissues or cells.

Gao, D., et al. (2009). "Metallothionein-2 gene from the mandarin fish Siniperca chuatsi: cDNA cloning, tissue expression, and immunohistochemical localization." Comp Biochem Physiol C Toxicol Pharmacol 149(1): 18-25.

The metallothionein-2 (MT-2) gene was isolated from the mandarin fish, one of the most important industrial aquatic animals in China, by using rapid amplification of cDNA ends (RACE). The deduced amino acid sequence of MT-2 comprised 60 amino acids and showed approximately 62.3% identity to human metallothionein. Its promoter region was amplified by thermal asymmetric interlaced polymerase chain reaction (TAIL-PCR). The MT-2 gene consists of 3 exons and 2 introns, extending approximately 900 bp of genomic sequence. Phylogenetic analysis clearly demonstrated that MT-2 formed a clade with fish metallothionein. The promoter region contained 5 putative metal-regulatory elements (MREs) and 1 TATA box. Real-time quantitative RT-PCR analysis revealed that MT-2 transcripts were significantly increased in the brain and gills and were stable in the muscles, liver, and trunk kidney in Cd(2+)-stimulated fish. Western blotting analysis demonstrated that the protein of the MT-2 gene was expressed mainly in the gills, liver, heart, trunk kidney, muscle, and intestine; it was weakly detected in the brain and head kidney. Moreover, the MT-2 protein was immunohistochemically detected in the cytoplasm in the liver and trunk kidney. All the above results revealed that the mandarin fish MT-2 would be a useful biomarker for metal pollution.

Garg, L. C., et al. (1989). "Interaction of a positive regulatory factor(s) with a 106-base pair upstream region controls transcription of metallothionein-I gene in the liver." J Biol Chem 264(4): 2134-2138.

The differential transcription of the cloned mouse metallothionein-I (MT-I) gene in tissues was studied in unfractionated and fractionated nuclear extracts from rat liver and brain. MT-I gene transcription was 10-fold greater in liver nuclear extract than in brain nuclear extract, whereas the level of transcription of the histone H4 gene was almost identical in both tissue extracts. 5' Deletion analysis of upstream sequences revealed that a 106-base pair (bp) region located between the -148- and -42-bp positions with respect to the transcription start site was responsible for the higher level of expression of the MT-I gene in the liver. Preincubation of the liver extract with the 106-bp fragment resulted in a significant decrease in MT-I gene transcription in the liver extract. In contrast, MT-I gene transcription in the brain nuclear extract was not altered by preincubation with the 106-bp upstream sequence. Mixing liver and brain extract did not diminish MT-I gene transcription normally occurring in liver nuclear extract. Preincubation of brain nuclear extract with the MT-I gene had no inhibitory effect on transcription of MT-I gene in liver nuclear extract. These studies suggest that neither an inhibitor nor a negative trans-acting factor in the brain is responsible for the differential transcription of MT-I gene; rather a positive regulatory factor(s) in the liver which interacts with the 106-bp upstream region contributes to the higher level of MT-I gene expression in this tissue.

Garrett, S. H., et al. (2001). "Acute exposure to arsenite induces metallothionein isoform-specific gene expression in human proximal tubule cells." J Toxicol Environ Health A 64(4): 343-355.

The expression of metallothionein (MT) mRNA and protein was determined in human proximal tubule cells (HPT) following acute exposure to the classic stimulators of the stress response, heat and sodium arsenite (As3+). Treatment of the cells with 100 microM As3+ for 4 h resulted in a significant increase in the MT-1 and MT-2 proteins immediately preceding and following removal of the stress. The level of the MT-3 isoform protein was unchanged as a result of As3+ treatment. An analysis of the MT isoform-specific mRNA demonstrated that control cells express the MT-1E, MT-1F, MT-1X, MT-2A, and MT-3 genes, but not the MT-1A, MT-1B, MT-1C, MT-1H, and MT-4 genes. Treatment with As3+ resulted in a significant increase in the expression of the MT-1X gene and appearance of mRNA for the MT-1A gene. Expression of the other MT genes was unaffected by As3+ exposure, except one isolate expressed a low level of MT-1G mRNA at several time points. It is likely that the increase in MT protein seen in As3+-treated cells is due to the increased expression of the MT-1X gene because its expression is much greater than the MT-1A isoform. Treatment of the HPT cells with heat shock had no marked effect on the levels of MT protein or mRNA. This study demonstrates that acute exposure to As3+ increases the levels of MT protein and that this elevation most likely arises from increased expression of the MT-1X isoform.

Garrett, S. H., et al. (2000). "Metallothionein isoform 1 and 2 gene expression in the human prostate: downregulation of MT-1X in advanced prostate cancer." Prostate 43(2): 125-135.

BACKGROUND: Studies have shown an association of metallothionein (MT) overexpression with tumor type and grade. However, a family of genes underlies the expression of these proteins. The goals of this study were to define the expression of MT genes and protein in normal human prostate and to provide evidence that the expression of the MT isoforms is altered in prostate cancer. METHODS:

Immunohistochemistry was used to localize MT protein, reverse transcription-polymerase chain reaction (RT-PCR) to determine the MT isoform-specific mRNAs, and immunoblot analysis to determine MT protein levels. RESULTS: The localization of MT in the prostate was further defined using the E9 antibody. Using normal prostate tissue dissected from glands removed for prostate cancer, it was demonstrated that MT protein expression in the normal prostate is supported by mRNA from the MT-1A, MT-1E, MT-1X, and MT-2A genes. No expression of the MT-1X gene was demonstrated in cases of advanced prostate cancer. The expression of MT-1 and MT-2 isoform-specific mRNA varied among three commonly utilized prostate cancer cell lines. CONCLUSIONS: MT protein in the normal human prostate is supported by transcription of mRNA from the MT-1A, MT-1E, MT-1X, and MT-2A genes. Expression of MT-1X mRNA is downregulated in advanced prostate cancer. Variable expression of MT mRNA in prostate cell lines provides evidence that MT gene expression may be altered among individual prostate cancers.

Garte, S. J., et al. (1995). "Kinetics of metallothionein gene induction by cadmium in human lymphocytes." Biochem Mol Biol Int 37(3): 459-465.

Induction of gene transcription is a complex process involving a diverse set of transcription factors and regulatory steps. We have taken a kinetic approach to analysis of metallothionein gene induction in human peripheral blood lymphocytes. By repeated measurements of MT mRNA after incubation of cells in vitro with CdCl2, we were able to determine individual-specific time related constants. The kinetics of induction for 3 individuals followed an S shaped curve and the data was fitted to a modified kinetic model of gene transcription. From this model, which assumes a cooperativity effect, transcriptional and RNA degradation rate constants could be calculated. The rate constant for transcription was doubled with the doubling of CdCl2 concentration, but the rate constant for RNA degradation was independent of Cd concentration.

Gautam, N., et al. (2012). "Genome-wide identification of rice class I metallothionein gene: tissue expression patterns and induction in response to heavy metal stress." Funct Integr Genomics 12(4): 635-647.

Metallothioneins (MTs) are members of a family of cysteine-rich low molecular weight polypeptides which play an important role in heavy metal detoxification and homeostasis of intracellular metal ions in plant. Though MT genes from some selected plants have been characterized with respect to their protein sequences, kinetic properties and tissue-specific localization, no detailed study has been carried out in rice. Here, we present genome-wide identification, structural and expression analyses of rice MT gene family. Our analysis suggests presence of 11 class I MT genes in rice genome (Release 7 of the MSU Rice Genome Annotation Project) which are differentially expressed during growth and development, in various tissues and during biotic and abiotic stresses. Our analyses suggest that class I MT proteins in rice differ in tissue localization as well as in heavy metal coordination chemistry. We also suggest that some MTs have a predominant role in detoxification of As (V) in arsenic-tolerant rice cultivars. Our analysis suggests that apart from transcriptional regulation, post-transcriptional alternative splicing in some members of this family takes place during growth and development, in various tissues and during biotic and abiotic stresses.

Gholap, P. N., et al. (2016). "Screening the partial coding region of metallothionein isoform-2 gene in Zebu cattle." Iran J Vet Res 17(3): 155-159.

Metallothionein (MT) is important because it binds tightly to heavy metals to decrease their toxicity. DNA was isolated from 30 toxic metal exposed and 30 toxic metal unexposed Zebu cows. The amplified metallothionein isoform-2 (MT-2) PCR product (489 bp) was further used for PCR-RFLP and DNA sequencing. MT-2 TaqI PCR-RFLP revealed homozygous genotype (AA) except for the E23 animal (AB). The genotype frequency of AA and AB (E23) genotypes in the exposed groups was 0.967 and 0.033 respectively. DNA sequencing was carried out for the toxic metal exposed sample (E23) and the control group sample (C13). Blast comparisons of the sequences were then aligned against a nucleotide database which revealed 150 nucleotide substitutions consisting of 70 transitions and around 80 transversions. DNA sequencing followed by PCR-RFLP for MT-2 revealed a higher number of nucleotide substitutions (150) for the AB genotype of E23 as compared to the AA genotype (38) of E21. The proportions of transversion mutations in the AB genotype were higher as compared to the MT-2 AA genotype. DNA sequencing was carried out based on random sampling for E21 and C13. Alignment analysis of the E21 and C13 sample revealed 38 nucleotide substitutions consisting of equal numbers of transition and transversion. BLAST analysis of the identified partial sequence revealed 89% identity with Bos taurus, 85% identity with sheep, 98% identity with buffalos and 100% identity with goat MT-2 sequences. Overall findings of the present study revealed DNA sequence variation in the coding region of the MT-2 gene of Zebu cattle which can be utilised to characterize and explore markers for heavy metal homeostasis in Zebu cattle.

Ghoshal, K. and S. T. Jacob (2001). "Regulation of metallothionein gene expression." Prog Nucleic Acid Res Mol Biol 66: 357-384.

The rapid and robust induction of metallothioneins (MT)-I and II by a variety of inducers that include heavy toxic metals, reactive oxygen species, and different types of stress provide a useful system to study the molecular mechanisms of this unique induction process. The specific expression of MT-III in the brain and of MT-IV in the squamous epithelium of skin and tongue offers a unique opportunity to identify and characterize the tissue-specific factors involved in their expression. Studies using transgenic mice that overexpress MTs or MT null mice have revealed the role of MT in the protection of cells against numerous tissue-damaging agents such as reactive oxygen species. The primary physiological function of these proteins, however, remains an enigma. Considerable advances have been made in the identification of the cis-acting elements that are involved in the constitutive and induced expression of MT-I and MT-II. By contrast, only one key trans-activating factor, namely MTF-1, has been extensively characterized. Studies on the epigenetic silencing of MT-I and MT-II by promoter hypermethylation in some cancer cells have posed interesting questions concerning the functional relevance of MT gene silencing, the molecular mechanisms of MT suppression in these cells, particularly chromatin modifications, and the characteristics of the repressors.

Giritch, A., et al. (1998). "Structure, expression and chromosomal localisation of the metallothionein-like gene family of tomato." Plant Mol Biol 37(4): 701-714.

Metallothioneins are small cysteine-rich proteins with strong binding capacity for heavy metals. In animals and fungi they are involved in cellular detoxification processes. Although genes for similar proteins exist in plants, less is known about the putative functions of their protein products. Here, we describe the characterisation of cDNAs specific for four genes (LEMT1, LEMT2, LEMT3 and LEMT4) encoding metallothionein-like proteins from tomato. Based on the characteristic cysteine pattern, the LEMT1, LEMT3 and LEMT4 gene products represent type 2 proteins. In contrast, the LEMT2 protein might establish a new structural pattern of metallothionein-like proteins not described before. Mapping experiments demonstrate that all four genes are localised at different genetic loci within the tomato genome. The members of the small gene family show a differential organ specific expression pattern. Expression of these genes is also influenced by heavy metals and by treatment with the thiol-oxidising drug diamide. We further describe the expression of the LEMT genes under different iron supply conditions both in tomato wild type as well as in the mutant chloronerva, which is defective in metal uptake regulation and exhibits a characteristic 'apparent iron deficiency syndrome'.

Glanville, N., et al. (1981). "Structure of mouse metallothionein-I gene and its mRNA." Nature 292(5820): 267-269.

Metallothioneins are small cysteine-rich proteins that bind heavy metals such as zinc, cadmium, copper and mercury. Recent interest in these proteins has focused on the part they play in zinc metabolism and heavy metal detoxification. Our interest in metallothionein genes stems largely from the observations that these proteins are inducible by both heavy metals and glucocorticoid hormones. To explore the regulation of these genes, we have isolated cDNA and genomic clones corresponding to mouse metallothionein-I (MT-I), and have used them to show that both inducers act at the transcriptional level in vivo and in a wide variety of cell lines. We have also shown that the MT-I gene is amplified during selection for cadmium resistance. To investigate the mechanisms of gene regulation, knowledge of the primary DNA sequence is necessary. Here we present the entire sequence of mouse MT-I gene along with approximately 300 bases of 5' flanking region that presumably includes promoter and regulatory sites. The 5' mRNA sequence, defined by S1 nuclease mapping, was combined with sequences of the coding and 3' untranslated regions obtained previously to allow a computer prediction of the most stable secondary structure of MT-I mRNA.

Gong, L., et al. (2015). "Cloning, characterization, and expression of cadmium-induced metallothionein-2 gene from earthworm Pheretima aspergillum (E. Perrier)." Genet Mol Res 14(4): 16782-16792.

Metallothioneins (MTs) are ubiquitous metal-binding, cysteine-rich proteins, associated with metal accumulation and thus providing protection against toxic heavy metals such as cadmium (Cd). To investigate the mechanisms of enrichment of Cd in the earthworm Pheretima aspergillum, we isolated and cloned metallothionein-2 (MT-2) cDNA (538 bp) from P. aspergillum, analyzed its sequence, and examined MT-2 transcription levels by relative quantitative real-time PCR under different concentrations of Cd. The sequence of P. aspergillum MT-2 cDNA and its putative amino acid sequence were highly similar to sequences from other earthworms. The induction with Cd increased the MT-2 gene transcription level in a dose-dependent manner. In addition, earthworm recombinant MT-2 exhibited high Cd bioaccumulation ability in vitro. These results suggested that MT-2 plays an important role in tolerance and accumulation of Cd in P. aspergillum.

Gong, W., et al. (2019). "The effect of CTCF binding sites destruction by CRISPR/Cas9 on transcription of metallothionein gene family in liver hepatocellular carcinoma." Biochem Biophys Res Commun 510(4): 530-538.

Chromatin spatial organization is essential for

transcriptional modulation and stabilization. The pattern of DNA distal interplay form the multiple topological associating domains (TADs), and further assemble the functional compartmentalization with open and expression-active chromatin ("A" compartments) or closed and expression-inactive chromatin ("B" compartments) in genome, whose boundaries were defined by the high enrichment of CCCTC-binding factor (CTCF). Nevertheless, As a potential therapeutic strategy, changing the local chromatin architecture via adding or removing the CTCF binding sites in situ to regulate the transcription activity of genes within one TAD in cancer cells is poorly explored. In present study, we observed that the metallothionein (MT) family were all remarkably decreased in HCC of TCGA database, and MT genes family were located within a TAD of 1.2Mbat 16q13 in order, and CTCF binding sites were distributed at the both sites of MT gene clusters. Furthermore, CRISPR/Cas9 was employed to destroy the CTCF binding sites at the vicinity of the MT family in human liver hepatocellular carcinoma (HCC) cell lines Huh-7 and HepG2. And the presence of up-regulated transcription of MTs were observed in Huh-7 and HepG2 cells compared to normal liver CRL-12461cells. Moreover, the presence of the varying DNA interplay as well as H3K4me3 and H3K9me3 modification on different MT genes were observed after CTCF binding domain destruction compared to the control using chromosome conformation capture (3C) and chromatin immunoprecipitation (ChIP). Our results determined a potential way to regulate the transcription of a series of genes via changing the local genomic organization for diseases treatment.

Gorman, J. A., et al. (1986). "Regulation of the yeast metallothionein gene." Gene 48(1): 13-22.

To study regulation of the yeast CUP1 gene, we have employed plasmids containing the CUP1 regulatory sequences fused to the Escherichia coli galK gene. A comparison of galK expression from low- and high-copy-number CUP1/galK fusion plasmids demonstrated that both basal and induced levels of galactokinase (GalK) increase proportionately with plasmid copy number. Host strains with an amplified, single or deleted CUP1 locus were compared to look for effects of chromosomal CUP1 gene dosage on expression from the episomal CUP1 promoter. Basal GalK levels are similar in CUP1R and cupls hosts, but can be induced to higher levels in the cup1s than the CUP1R host. In contrast, in a strain deleted for the chromosomal copy of CUP1, synthesis of GalK is constitutive but can be induced to yet higher levels by copper. A hybrid vector, placing the CUP1 coding sequence under the control of a constitutive promoter, was constructed. Introduction of this hybrid CUP1 gene into the deletion host containing the CUP1/galK plasmid restores regulation. Thus, metallothionein, in trans, can effect repression of the CUP1 promoter. The possible roles of metallothionein and free copper in CUP1 regulation are discussed.

Habener, J. F., et al. (1989). "Metallothionein-vasopressin fusion gene expression in transgenic mice. Nephrogenic diabetes insipidus and brain transcripts localized to magnocellular neurons." J Biol Chem 264(31): 18844-18852.

Arginine vasopressin (AVP) is a potent neuroactive and vasoactive nonapeptide encoded in and processed from a precursor, preproarginine vasopressin-neuro-physin II (preproAVP-NPII). To study the physiologic consequences of a genetic model of chronic hypervasopressinemia transgenic mice were produced by introduction of a mouse metallothionein-rat-ppAVP-NPII fusion gene into the germ line of mice. Three stable transgenic pedigrees were analyzed through several generations. Levels of immunoreactive AVP and neurophysin (NP) in sera, livers, kidneys, intestines, pancreas, and brains were markedly elevated. Chromatographic analyses showed sera levels of approximately 500 pg/ml (normal 0-20 pg/ml) of authentic AVP non-apeptide and serum osmolalities were elevated, 315.4 +/- 1.4 mosm/liter (control, 307.3 +/- 1.1), consistent with a state of mild nephrogenic diabetes insipidus. Brain levels of immunoreactive AVP in transgenic mice were 3-4-fold elevated 145 +/- 15 ng/g versus 31 +/- 7 (controls). Although immunoreactive AVP in livers and intestines, and to some extent kidneys, consisted predominantly of unprocessed precursors, in brain and pancreas greater than 90% of AVP consisted of processed bioactive nonapeptide, as determined by chromatography and measurements of cAMP-generation in LLC-PK1 cells. Immunocytochemistry localized immunoreactive AVP to the exocrine pancreas and to the magnacellular neurons (SON and PVN) of the hypothalamus. Expression of the fusion gene in the hypothalamus was further demonstrated by Northern analyses of fusion gene specific transcripts and in situ histohybridization. Although the fusion gene contained only 35 base pairs of 5'-flanking DNA of the ppAVP-NPII gene, a tentative neuronal cell-specific expression element, -17GCCCAG-CC-10 resides in this sequence and may confer neuron-specific expression to the fusion gene.

Hadama, T., et al. (2002). "Endogenous tumor necrosis factor promotes resistance to cellular stresses by inducing the metallothionein-1A gene." Anticancer Res 22(6C): 4059-4063.

BACKGROUND: Endogenous tumor necrosis factor (enTNF) acts as a resistance factor against anticancer drugs, heat and exogenous TNF via induction of manganous superoxide dismutase (MnSOD) and heat shock protein 72 (HSP72), while the details of interaction with other molecules are not

fully understood. We compared mRNA expression of various genes between MIAPaCa-2 human pancreatic cancer cells and M5 cells transduced with nonsecretory-type TNF gene expression vector. MATERIALS AND METHODS: The expression of certain mRNAs between human pancreatic cancer MIAPaCa-2 cells and M5 cells was compared using fluorescent differential display. RESULTS: Of 140 bands obtained by gel electrophoresis, 53 bands showed patterns differing between MIAPaCa-2 and M5 cells. Among these bands, sequence analysis and RT-PCR identified strong mRNA expression for metallothionein-1A, a scavenger of reactive oxygen species, in transduced M5 cells. CONCLUSION: Metallothionein-1A could be induced by enTNF, resulting in resistance against various cellular stresses.

Hayashi, K., et al. (1983). "Effects of tumor promoters on the frequency of metallothionein I gene amplification in cells exposed to cadmium." Cancer Res 43(11): 5433-5436.

Three potent tumor promoters of different classes, 12-O-tetradecanoylphorbol-13-acetate, dihydroteleocidin B, and aplysiatoxin, and two moderate tumor promoters, mezerein and debromoaplysiatoxin, enhanced the frequency of appearance of cadmium-resistant Chinese hamster lung cells when the cells were exposed to cytotoxic levels of CdCl2. With these compounds, the activity to induce cadmium-resistant cells correlated well with the potency of tumor-promoting activity. Cadmium resistance, which persisted after removal of the tumor promoters, was associated with the overproduction of metallothionein I messenger RNA. The amplified metallothionein I genes were shown by Southern blotting experiments. The relevance of the gene amplification caused by tumor promoters is discussed in relation to cancer development and progression.

Haynes, V., et al. (2013). "Metallothionein 2a gene expression is increased in subcutaneous adipose tissue of type 2 diabetic patients." Mol Genet Metab 108(1): 90-94.

STUDY BACKGROUND: Insulin resistance plays an important role in the pathogenesis of type 2 diabetes and the metabolic syndrome. Many of the genes and pathways involved have been identified but some remain to be defined. Metallothioneins (Mts) are a family of anti-oxidant proteins and metallothionein 2a (Mt2a) polymorphims have been recently associated with type 2 diabetes and related complications. Our objective was to determine the Mt2a gene expression levels in adipose tissues from diabetic patients and the effect of Mt treatment on adipocyte insulin sensitivity. METHODS: Samples of subcutaneous and visceral adipose tissues from lean, type 2 diabetic and non-diabetic obese patients were analysed using RT-qPCR for Mt2a mRNA abundance. The regulation of Mt2a expression was further studied in 3T3-L1 adipocytes treated or not with TNFalpha (10 ng/ml, 72 h) to induce insulin resistance. The effects of Mt on glucose uptake were investigated in cultured adipocytes treated with recombinant Mt protein. RESULTS: We found that the Mt2a gene expression was significantly higher in adipose tissue of type 2 diabetic patients in comparison to that of lean (p=0.003) subjects. In 3T3-L1 adipocytes, insulin resistance induced by TNFalpha increased Mt2a mRNA levels (p=3x10(-4)) and insulin-stimulated glucose uptake was significantly inhibited by 53% (p=8x10(-4)) compared to vehicle, when 3T3-L1 adipocytes were treated with Mt protein. CONCLUSIONS: These data suggest that Mt2a might be involved in insulin resistance through the up-regulation of Mt gene expression, which may lead to the modulation of insulin action in fat cells. These results suggest the concept of considering Mt proteins as markers and potential targets in type 2 diabetes.

He, P., et al. (2007). "Cloning and functional characterization of 5'-upstream region of metallothionein-I gene from crucian carp (Carassius cuvieri)." Int J Biochem Cell Biol 39(4): 832-841.

Metallothioneins are low molecular weight, cysteine-rich, metal-binding proteins, which can be induced by heavy metal ions, cytokines, stress, and hormones. To investigate the roles of the main cis-acting elements involved in the inducible expression of metallothionein gene in fish, the 5'-upstream region of crucian carp (Carassius cuvieri) metallothionein-I gene had been cloned and analyzed after our previous work on metallothionein-II. In its upstream region, several putative cis-acting elements, including nine metal regulatory elements (MREs), one antioxidant response element, one E-box, and three interleukin-6 responsive elements, etc. were found. The nine metal regulatory elements are confined in less than 1000 bp from ATG start codon and organized into two clusters with different roles to the induction of the metallothionein-I expression. Deletion mutant assays demonstrated that both the distal and proximal clusters of metal regulatory elements contributed to the basal expression of the metallothionein-I, but only the proximal cluster was the chief contributor to the metal fold induction. In transient luciferase reporter assays, Zn2+ and Cd2+ served as much stronger inducers than Cu2+ to the metallothionein-I expression. H2O2 also could activate the metallothionein-I promoter about two-fold, which was mediated by the antioxidant response element (TGACAACGC, -437/-445). In conclusion, our studies demonstrate the roles of metal regulatory element and antioxidant response element in the induction of crucian carp metallothionein-I gene, and provide the regulatory mechanism for the use of fish metallothionein as a biomarker for monitoring of metal contamination in waters.

He, X., et al. (2000). "[Cloning and expression of metallothionein gene of Bombyx mori]." Wei Sheng Wu Xue Bao 40(4): 384-388.

Yeast MTI gene from vector pCMI-1 was used as a probe. It appeared strong hybridization signals when the total DNA of Bombyx mori Huishu eggs hybridizes with the probe. The 1-6 kb DNA fragments were isolated from the EcoR I digested total DNA of Bombyx mori Huishu eggs and ligated with M13-vector digested by restriction EcoR I. The ligation mixtures were used to transform E. coli DH5 alpha. blue/White colonies selection was used to identify colonies with insert. Approximately 4000 white colonies were selected, so the part genomic library of Bombyx mori was constructed. Three positive colonies were gained from the genomic library by southern blotting analysis, designated T1 (pZHC-1), T5 (pZHC-5), T7 (pZHC-7). Digesting the recombinant plasmid pZHC-5 with 12 restriction enzymes, the results suggested that the inserted fragment was about 1.2 kb and there was only a Hind III site. The experiment of resistant to CuSO4 proved that the DH5 alpha cells contain recombinant plasmids were more resistance than the recepient DH5 alpha cells. According to these results, the inserted fragment possibly contains the gene encoding Metallothionein of Bombyx mori. The sequence analysis of the inserted fragment and its high-expressions in E. coli are in progress.

Heguy, A., et al. (1986). "Structure and tissue-specific expression of the human metallothionein IB gene." Mol Cell Biol 6(6): 2149-2157.

The human metallothionein (MT) IB gene (hMT-IB) is located in a region of human DNA containing at least four tandemly arranged MT genes. As deduced from its sequence, hMT-IB is likely to encode a functional protein. However, the predicted amino acid sequence differed from the hMT-I amino acid sequence in four positions. Most remarkable was the presence of an additional cysteine. Like other MT genes, hMT-IB has at least two copies of the metal-responsive element upstream from the transcription initiation site. These elements probably are responsible for the metal responsiveness of the hMT-IB promoter, leading to inducible expression of fused heterologous genes. Unlike the hMT-IIA and hMT-IA genes described previously, which are expressed in many different cell types, a high level of expression of the endogenous hMT-IB gene could be detected only in human hepatoma and renal carcinoma cell lines. Therefore, this is the first MT gene described which exhibits tissue specificity of expression. This specificity is controlled by a cis-acting mechanism involving methylation, since incubation of nonexpressing cells with an inhibitor of DNA methylation led to activation of the hMT-IB gene. In support of this notion, we found that the 5' flanking region of the hMT-IB gene was highly methylated in HeLa cells, a nonexpressing cell type, but it was not methylated in a hepatoma (expressing) cell line.

Hempe, J. M., et al. (1991). "Intestinal metallothionein gene expression and zinc absorption in rats are zinc-responsive but refractory to dexamethasone and interleukin 1 alpha." J Nutr 121(9): 1389-1396.

The effects of dexamethasone and interleukin 1 alpha on intestinal metallothionein gene expression and zinc absorption were studied. Rats given parenteral zinc served as positive controls. A single intraperitoneal or intravenous dose of dexamethasone, interleukin 1 alpha or zinc markedly increased liver metallothionein synthesis 3-9 h after injection. Intestinal metallothionein mRNA and metallothionein protein were not affected by dexamethasone or interleukin 1 alpha, but were markedly increased by parenteral zinc. Absorption of 65Zn from isolated duodenal segments was inversely related to intestinal metallothionein concentration in rats given zinc, but was not affected by dexamethasone or interleukin 1 alpha. Plasma zinc concentrations decreased in rats given dexamethasone or interleukin 1 alpha and increased in those given zinc, but they were not related to 65Zn absorption. Similarly, multiple intraperitoneal administration of either dexamethasone or interleukin 1 alpha, or oral administration of dexamethasone, for 7 d markedly increased liver metallothionein synthesis but did not affect intestinal metallothionein concentration or 65Zn absorption. These results suggest that intestinal metallothionein gene expression and 65Zn absorption are refractory to glucocorticoid hormone and interleukin 1 alpha.

Hennigar, S. R., et al. (2021). "Sensitivity and reliability of zinc transporter and metallothionein gene expression in peripheral blood mononuclear cells as indicators of zinc status: responses to ex vivo zinc exposure and habitual zinc intake in humans." Br J Nutr 125(4): 361-368.

Zn is an essential nutrient for humans; however, a sensitive biomarker to assess Zn status has not been identified. The objective of this study was to determine the reliability and sensitivity of Zn transporter and metallothionein (MT) genes in peripheral blood mononuclear cells (PBMCs) to Zn exposure ex vivo and to habitual Zn intake in human subjects. In study 1, human PBMCs were cultured for 24 h with 0-50 microm ZnSO4 with or without 5 microm N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), and mRNA expression of SLC30A1-10, SLC39A1-14, MT1 subtypes (A, B, E, F, G, H, L, M and X), MT2A, MT3 and MT4 mRNA was determined. In study 2, fifty-four healthy male and female volunteers (31.9 (sd 13.8) years, BMI 25.7 (sd 2.9) kg/m2) completed a FFQ, blood was collected, PBMCs were isolated and mRNA

expression of selected Zn transporters and MT isoforms was determined. Study 1: MT1E, MT1F, MT1G, MT1H, MT1L, MT1M, MT1X, MT2A and SLC30A1 increased with increasing concentrations of Zn and declined with the addition of TPEN. Study 2: Average daily Zn intake was 16.0 (sd 5.3) mg/d (range: 9-31 mg/d), and plasma Zn concentrations were 15.5 (SD 2.8) mumol/l (range 11-23 mumol/l). PBMC MT2A was positively correlated with dietary Zn intake (r 0.306, P = 0.03) and total Zn intake (r 0.382, P < 0.01), whereas plasma Zn was not (P > 0.05 for both). Findings suggest that MT2A mRNA in PBMCs reflects dietary Zn intake in healthy adults and may be a component in determining Zn status.

Heuchel, R., et al. (1994). "The transcription factor MTF-1 is essential for basal and heavy metal-induced metallothionein gene expression." EMBO J 13(12): 2870-2875.

We have described and cloned previously a factor (MTF-1) that binds specifically to heavy metal-responsive DNA sequence elements in the enhancer/promoter region of metallothionein genes. MTF-1 is a protein of 72.5 kDa that contains six zinc fingers and multiple domains for transcriptional activation. Here we report the disruption of both alleles of the MTF-1 gene in mouse embryonic stem cells by homologous recombination. The resulting null mutant cell line fails to produce detectable amounts of MTF-1. Moreover, due to the loss of MTF-1, the endogenous metallothionein I and II genes are silent, indicating that MTF-1 is required for both their basal and zinc-induced transcription. In addition to zinc, other heavy metals, including cadmium, copper, nickel and lead, also fail to activate metal-responsive promoters in null mutant cells. However, cotransfection of an MTF-1 expression vector and metal-responsive reporter genes yields strong basal transcription that can be further boosted by zinc treatment of cells. These results demonstrate that MTF-1 is essential for metallothionein gene regulation. Finally, we present evidence that MTF-1 itself is a zinc sensor, which exhibits increased DNA binding activity upon zinc treatment.

Hirako, N., et al. (2014). "A PU.1 suppressive target gene, metallothionein 1G, inhibits retinoic acid-induced NB4 cell differentiation." PLoS One 9(7): e103282.

We recently revealed that myeloid master regulator SPI1/PU.1 directly represses metallothionein (MT) 1G through its epigenetic activity of PU.1, but the functions of MT1G in myeloid differentiation remain unknown. To clarify this, we established MT1G-overexpressing acute promyelocytic leukemia NB4 (NB4MTOE) cells, and investigated whether MT1G functionally contributes to all-trans retinoic acid (ATRA)-induced NB4 cell differentiation. Real-time PCR analyses demonstrated that the inductions of CD11b and CD11c and reductions in myeloperoxidase and c-myc by ATRA were significantly attenuated in NB4MTOE cells. Morphological examination revealed that the percentages of differentiated cells induced by ATRA were reduced in NB4MTOE cells. Since G1 arrest is a hallmark of ATRA-induced NB4 cell differentiation, we observed a decrease in G1 accumulation, as well as decreases in p21WAF1/CIP1 and cyclin D1 inductions, by ATRA in NB4MTOE cells. Nitroblue tetrazolium (NBT) reduction assays revealed that the proportions of NBT-positive cells were decreased in NB4MTOE cells in the presence of ATRA. Microarray analyses showed that the changes in expression of several myeloid differentiation-related genes (GATA2, azurocidin 1, pyrroline-5-carboxylate reductase 1, matrix metallopeptidase -8, S100 calcium-binding protein A12, neutrophil cytosolic factor 2 and oncostatin M) induced by ATRA were disturbed in NB4MTOE cells. Collectively, overexpression of MT1G inhibits the proper differentiation of myeloid cells.

Hockner, M., et al. (2015). "Metallothionein gene activation in the earthworm (Lumbricus rubellus)." Biochem Biophys Res Commun 460(3): 537-542.

In order to cope with changing environmental conditions, organisms require highly responsive stress mechanisms. Heavy metal stress is handled by metallothioneins (MTs), the regulation of which is evolutionary conserved in insects and vertebrates and involves the binding of metal transcription factor 1 (MTF-1) to metal responsive elements (MREs) positioned in the promoter of MT genes. However, in most invertebrate phyla, the transcriptional activation of MTs is different and the exact mechanism is still unknown. Interestingly, although MREs are typically present also in invertebrate MT gene promoters, MTF-1 is notably absent. Here we use Lumbricus rubellus, the red earthworm, to study the elusive mechanism of wMT-2 activation in control and Cd-exposed conditions. EMSA and DNase I footprinting approaches were used to pinpoint functional binding sites within the wMT-2 promoter region, which revealed that the cAMP responsive element (CRE) is a promising candidate which may act as a transcriptional activator of invertebrate MTs.

Holloway, A. F., et al. (1997). "Human metallothionein gene MT1L mRNA is present in several human tissues but is unlikely to produce a metallothionein protein." FEBS Lett 404(1): 41-44.

A human MT gene from the functional locus on chromosome 16, MT1L, is characterised and shown to produce mRNA in at least four human tissues. This gene is unlikely to produce a metallothionein protein because it contains a termination codon at position 26, by analogy to other human MT1 genes. MT1L cDNA is almost identical to another metallothionein cDNA

clone reported recently, MT1R, suggesting that either there are unmapped human metallothionein genes, or that MT1L is polymorphic.

Homa, J., et al. (2010). "Metal-specific effects on metallothionein gene induction and riboflavin content in coelomocytes of Allolobophora chlorotica." Ecotoxicol Environ Saf 73(8): 1937-1943.

Metal pollution affects earthworm coelomocytes, including their differential counts, riboflavin content and metallothioneins (MT) involved in metal homoeostasis and detoxification. The present work shows effects of Ni, Cu, Zn, Cd, and Pb at the same molarity (1mM) on coelomocytes of Allolobophora chlorotica after 2-day worm dermal exposure to metal chlorides. Numbers of coelomocytes/eleocytes extruded by electric shock and amounts of riboflavin in coelomocyte lysates were significantly decreased in Cu-exposed worms, less diminished in response to Ni, Zn, Cd, and unaffected by Pb. In sharp contrast, real-time PCR revealed a very strong (272 fold) MT-mRNA induction in response to Cd only. The induction was very low in response to Zn, Cu, Pb, and Ni ions (2.6, 2.1, 1.4, and 1.3-fold, respectively). In conclusion, decreased cell counts and riboflavin content are molecular biomarkers of Cu exposure while induction of MT-mRNA is a molecular biomarker of worm Cd exposure.

Homa, J., et al. (2015). "Dermal exposure of Eisenia andrei earthworms: Effects of heavy metals on metallothionein and phytochelatin synthase gene expressions in coelomocytes." Environ Toxicol Chem 34(6): 1397-1404.

Parameters such as total number of coelomocytes, riboflavin content in coelomocytes, expression of genes implied in metal homeostasis, and detoxification mechanisms can be used as biomarkers to assess the impact of metals on annelids. Defense biomarkers (detoxification gene expressions and coelomocyte parameters) were investigated in the ecotoxicologically important species Eisenia andrei following in vivo exposure to 5 different metals (zinc, copper, nickel, lead, and cadmium) at known concentrations. Coelomocyte numbers and riboflavin content were not affected by metallic exposure, but metal-specific gene expression variations were evidenced.

Hong, Y. and M. Schartl (1992). "Structure of the rainbow trout metallothionein A gene." Gene 120(2): 277-279.

To investigate the regulation of metallothionein-encoding genes in fish, we have isolated and sequenced the rainbow trout metallothionein-A-encoding gene (tMT-A) by polymerase chain reaction. This gene spans about 1.1 kb, consists of three exons and two introns, and has an A+T-rich 5'-region which contains a TATAAA signal, and two metal responsive elements (MREs). The transcription start point is centered around an A residue 81 nt upstream of the ATG codon.

Hottiger, T., et al. (1995). "2-micron vectors containing the Saccharomyces cerevisiae metallothionein gene as a selectable marker: excellent stability in complex media, and high-level expression of a recombinant protein from a CUP1-promoter-controlled expression cassette in cis." Yeast 11(1): 1-14.

We have constructed 2-micron-based yeast expression vectors containing a copy of the metallothionein (CUP1) gene of Saccharomyces cerevisiae as a semi-dominant, selectable marker. When used for the expression of the thrombin inhibitor hirudin, originally derived from the leech Hirudo medicinalis, these vectors displayed the following characteristics. (1) In the presence of copper salts, they were mitotically more stable than similarly designed control vectors lacking the CUP1 gene. In copper-sensitive host strains, the apparent plasmid stability was 100%, even in complex media and during fed-batch fermentation for an extended period of time. (2) Use of the CUP1-stabilized plasmids improved the production of hirudin by both copper-sensitive and copper-resistant hosts. The highest hirudin titers were obtained with a delta CUP1 host. (3) Copper selection resulted in a moderate increase in average plasmid copy numbers (up to two-fold) as assessed by measuring hirudin expression from a constitutive promoter (GAPFL). This effect was most noticeable if the vector showed an asymmetric segregation pattern (i.e., high rates of plasmid loss in the absence of copper). (4) The CUP1 marker proved particularly useful in combination with a CUP1-promoter-controlled expression cassette on the same plasmid. In such a set-up, the rates of transcription of the heterologous protein and that of the selectable marker are tightly linked. Therefore, an increase in selective pressure directly provokes an increase in product yields. In a copper-sensitive host strain, this plasmid design allowed for the production of very high amounts of biologically active hirudin. Our results clearly establish the utility of the CUP1 marker in the construction of stable yeast expression vectors.

Hou, Y. M., et al. (1988). "Expression of the mouse metallothionein-I gene in Escherichia coli: increased tolerance to heavy metals." Biochim Biophys Acta 951(1): 230-234.

The cDNA of mouse metallothionein, a small metal-binding protein rich in cysteine, has been cloned downstream from a bacterial inducible promoter and expressed in Escherichia coli. Upon induction, E. coli harboring this cDNA clone contained a protein species readily labelled by [35S]cysteine in vivo and incorporated 10-times as much 109Cd from the medium than would otherwise be the case. We show

that expression of metallothionein endows resistance in E. coli to heavy metal ions such as mercury, silver, copper, cadmium and zinc by sequestering rather than exclusion or conversion, common mechanisms of metal resistance in bacteria.

Houben, R., et al. (1997). "Differential gene expression in apoptotic 32Dcl3 cells: induction of metallothionein." Apoptosis 2(1): 40-46.

Growth factor deprivation induced cell death of the hematopoietic cell line 32Dcl3 is widely used as a model system to study apoptotic signalling pathways. Here we show that the onset of cell death after IL-3 withdrawal can be strongly delayed by either cycloheximide or actinomycin D, indicating that de novo protein synthesis is required. Subtractive cDNA library hybridization was used to identify genes upregulated in apoptotic 32Dcl3 cells. Here we present data showing metallothionein-I (MT-I) mRNA transiently upregulated by a factor of three- to 20-fold. Increased levels of total MT-I+II protein after IL-3 withdrawal were demonstrated. An induction of MT-I RNA as well as of MT-I+II total protein was also observed in serum deprived NIH3T3 fibroblasts. Testing the effect of different inducers of apoptosis on 32Dcl3 cells we found that only IL-3 withdrawal and ethanol treatment led to an upregulation of MT-I mRNA level. Since MTs are believed to play a role in the metabolism of zinc, we tested the effect of zinc on induced cell death. When 32Dcl3 cells are treated with zinc (50-300 microM) in the absence of IL-3, loss of viability as well as degradation of the cellular DNA were delayed, indicating that zinc represses apoptosis. On the other hand zinc pre-treatment induced MT expression and accelerated the onset of apoptosis. Our data, therefore, suggest that MT exerts a proapoptotic function.

Hsieh, H. M. and P. C. Huang (1998). "Promoter structure and activity of type 1 rice metallothionein-like gene." DNA Seq 9(1): 9-17.

A novel stress-inducible metallothionein-like gene from rice, designated as rgMT-1 (rice genomic metallothionein-like gene-1), was isolated and sequenced. From the sequence analysis of its 5'-flanking region, two putative TATA boxes, one CAAT box, and several short sequences homologous to regulatory cis-elements previously reported were identified. Two direct repeats, one 10 bp in length (CAAAATCAAA) and the other 11 bp (GTGAAAATACT), respectively, were also found. By transient GUS (beta-glucuronidase) assay, the expression of GUS, in vitro, was enhanced by the presence of the rgMT-1 intron. The critical region which controls the basal transcription was shown to lie between -73 and -36 upstream of rgMT-1, in which one of the two putative TATA boxes was located. The promoter activity was lost completely when both putative TATA boxes were deleted. This is the first report describing the genomic structure and regulation of a monocotyledonous metallothionein-like gene critical to the response of stress.

Hsieh, H. M., et al. (1996). "RNA expression patterns of a type 2 metallothionein-like gene from rice." Plant Mol Biol 32(3): 525-529.

A type 2 metallothionein-like gene from rice, OsMT-2 (Oryza sativa metallothionein-like gene-2), was isolated in its cDNA form and sequenced. By northern analyses OsMT-2 expression was shown to be induced under stress by sucrose starvation, heat shock and, to a lesser extent, abscisic acid, but not excess metals, including copper. Its response to sucrose starvation was transient and different from OsMT-1, a type 1 metallothionein-like gene of rice inducible by copper. These results suggest that while OsMT-2 is also involved in cellular response to stress, its function may be complementary to that of OsMT-1.

Hsieh, H. M., et al. (1995). "A novel stress-inducible metallothionein-like gene from rice." Plant Mol Biol 28(3): 381-389.

A novel rice genomic sequence encoding coding segments homologous to other metallothionein-like genes was isolated from Oryza sativa genomic library. This sequence, hereby designated as rgMT (rice genomic metallothionein-like gene), consists of two exons and one intron. From the coding sequence, it is predicted that rgMT encodes one protein of 74 amino acids. Differential expression of rgMT in rice plants was observed as mature transcripts were more abundant in roots than in leaves and sheaths. Under different stress conditions, such as excess heavy metals and heat shock, expression of rgMT was significantly elevated. This was especially noticeable with 250 microM CuCl2 for 16 h, 40 degrees C heat for 2 h and 0.06% DMSO for 1 h. Under sucrose starvation, rgMT transcripts also increased with time up to 72 h. During recovery from sucrose starvation, the transcripts declined slightly within 12 h of recovery. rgMT transcripts were also seen to have increased expression in senescent leaves. These results support the notion that rgMT is a stress-inducible gene in rice heretofore unreported.

Hu, M. C. and N. Davidson (1990). "A combination of derepression of the lac operator-repressor system with positive induction by glucocorticoid and metal ions provides a high-level-inducible gene expression system based on the human metallothionein-IIA promoter." Mol Cell Biol 10(12): 6141-6151.

We and others have introduced the use of the lac operator-repressor system as a method for providing inducible gene expression for gene transfer experiments in animal cells (M. C.-T. Hu, and N. Davidson, Cell 48:555-566, 1987; J. Figge, C. Wright, C. J. Collins, T. M. Roberts, and D. M. Livingston, Cell

52:713-722, 1988). To improve the dynamic range of such an inducible system, we have investigated the effects of combining the relief by isopropyl-beta-D-thiogalactoside (IPTG) of negative control by the lac system with positive induction by the natural inducers glucocorticoids and cadmium ion for a system based on the human metallothionein-IIA gene promoter. We used the chloramphenicol acetyltransferase gene as a reporter gene and inserted a lacO sequence into the promoter between the GC box and metal-responsive element 1, between metal-responsive element 1 and the TATA box, or between the TATA box and the transcription start site. Surprisingly, all of these insertions had a significant inhibitory effect on promoter activity even in the absence of repressor. However, with these lacO-containing constructs, the levels of gene expression after induction by glucocorticoid, Cd2+, or both were considerably reduced in cells engineered to express the lac repressor. Derepression by IPTG, coupled with induction by both dexamethasone and Cd2+ ion, then provided a high level of induced expression, i.e., by a factor of approximately 100 over the basal level of expression. However, inserting the lacO sequence well upstream just before the glucocorticoid-responsive element had much smaller effects on expression levels in both repressor-negative and repressor-positive cells. This study describes a new, high-level-inducible promoter system for gene transfer experiments. The observed effects are discussed in terms of current models of the mechanisms by which transcription factors control gene expression.

Huang, G. Y. and Y. S. Wang (2009). "Expression analysis of type 2 metallothionein gene in mangrove species (Bruguiera gymnorrhiza) under heavy metal stress." Chemosphere 77(7): 1026-1029.

In this paper, we aimed to assess the roles of metallothioneins (MTs) in heavy metal tolerance by analyzing the expression level of BgMT2 in leaves of Bruguiera gymnorrhiza in response to heavy metals. Eight-month-old B. gymnorrhiza seedlings were exposed to different concentrations of zinc (Zn), copper (Cu) or lead (Pb) for 1, 3 and 7 d. A Real-time quantitative PCR protocol was developed to directly evaluate the expression of BgMT2, using 18S rRNA as a reference gene. Real-time quantitative PCR analysis demonstrated BgMT2 mRNA expression was regulated by Zn, Cu and Pb, but the regulation pattern was different for the three metals tested. Significant increase in the transcript level of BgMT2 was also found in response to Zn, Cu and Pb in some experimental conditions. Our results confirm that BgMT2 gene is involved in the regulation of Zn, Cu and Pb in B. gymnorrhiza leaves.

Huber, K. L. and R. J. Cousins (1988). "Maternal zinc deprivation and interleukin-1 influence metallothionein gene expression and zinc metabolism of rats." J Nutr 118(12): 1570-1576.

The influence of maternal dietary zinc intake and recombinant human interleukin-1 alpha (rhIL-1 alpha) administration on metallothionein gene expression and the distribution of 65Zn were investigated. Pregnant rats were fed diets containing 1, 5, 30 or 180 mg Zn/kg diet in an equalized regime from d 13-20 of gestation. Metallothionein gene expression was examined by Northern blot and dot blot hybridization using combined 60-mer oligonucleotides specific for rat metallothionein-1 and -2 genes. Expression was progressively depressed in the fetal livers and livers and kidneys of dams fed diets marginal (5 mg/kg) and deficient (1 mg/kg) in zinc content. Administration of rhIL-1 alpha increased expression in maternal liver, placenta and in fetal liver of dams fed adequate or deficient diets. Kinetics of intravenously administered 65Zn showed that in response to rhIL-1 alpha, there was a higher uptake by the maternal liver and bone marrow with less 65Zn uptake by bone, intestine and plasma activity compared to controls. No change was observed in 65Zn taken up by the placenta or transferred to the fetus. Alteration of metallothionein gene expression could represent, in part, the mechanism whereby altered effects of zinc metabolism and function are mediated during fetal development.

Hung, S. H., et al. (1991). "Molecular cloning of Chinese hamster metallothionein II gene and its 5' flanking region." Biochim Biophys Acta 1090(2): 255-258.

A genomic DNA clone containing Chinese hamster metallothionein II (MTII) gene and its 5' flanking region was isolated from Cd resistant Chinese hamster ovary (CHO) cells. DNA sequence analysis showed that there are three exons and two introns in the structure of the MTII gene. Further characterization of the 5' flanking region reveals the possible transcription initiation site, metal responsive element and basal-level enhancer sequence. Putatively, this is the promoter region of CHO MTII gene.

Hurko, O., et al. (1986). "Transfection of human skeletal muscle cells with SV40 large T antigen gene coupled to a metallothionein promoter." Ann Neurol 20(5): 573-582.

We have undertaken to increase the proliferative capacity of cultured human skeletal myocytes by transfection with a plasmid construct that contains the immortalizing and transforming large T antigen gene of simian virus 40 (SV40) under the control of a zinc-sensitive metallothionein promoter. This construct was chosen to permit rapid growth of transformants in zinc-containing medium, which induces high levels of T antigen expression, and muscle-specific differentiation after withdrawal of

exogenous zinc, which reduces levels of T antigen. When grown in 100 microM Zn2+, transformed myocytes expressed the large T antigen, divided rapidly, and acquired an apparently unlimited proliferative capacity. Transfer of these cells to a zinc-poor medium resulted in decreased T antigen immunofluorescence, growth rate, and saturation density as well as a return to a physiological spindle morphology. Despite transformation, these cells expressed differentiation markers characteristic of myoblasts: the B isoform of creatine kinase, and surface antigens 5.1H11, D5, and Thy 1 in the presence or absence of Zn2+. When grown to high density in a serum-poor medium, these cells differentiated further into typical multinucleated myotubes that expressed the M isoform of creatine kinase and increased levels of surface antigen 5.1H11, creatine kinase, and nicotinic acetylcholine receptors, but no detectable Thy 1 antigen. The specific activity of these differentiation markers was higher when the cells were grown in the absence of added zinc. These results indicate that transformation of human skeletal myocytes with a regulatable SV40 large T antigen gene allows an increase of the proliferative capacity of these cells with preservation of their capacity to differentiate in a physiological manner.

Iwata, M., et al. (1999). "Zinc accumulation and metallothionein gene expression in the proliferating epidermis during wound healing in mouse skin." Histochem Cell Biol 112(4): 283-290.

Metallothionein (MT), a low molecular weight metal-binding protein, has been related to zinc and copper metabolism, the acute-phase response, and cellular proliferation. In this study, we investigated changes in zinc metabolism and MT gene expression occurring in tissue damage and repair during wound healing in mouse skin. Northern blot analysis revealed that a significant increase of MT mRNA was observed in the liver for 18 h after wounding, and serum zinc downfall and hepatic zinc uptake were observed. In situ hybridization analysis showed that no significant expression of MT mRNA was detected within the first 9 h after wounding. However, it was expressed restrictively in the proliferating epidermis of the wound margin after 12 h. Zinc began to accumulate in wounded skin after MT gene expressed. Northern blotting and immunocytochemical staining revealed that MT has been synthesized actively during the growth phase compared with the stationary phase in normal human epidermal keratinocytes in vitro. Intracellular zinc accumulation was observed in the proliferating cells. We concluded that hepatic MT plays an important role as an acute phase protein against host damage, and epidermal MT contributes in the supply of zinc to wounded tissue and activates proliferation for the regeneration of epidermis.

Jadhav, R. R., et al. (2015). "Genome-wide DNA methylation analysis reveals estrogen-mediated epigenetic repression of metallothionein-1 gene cluster in breast cancer." Clin Epigenetics 7: 13.

BACKGROUND: Recent genome-wide analysis has shown that DNA methylation spans long stretches of chromosome regions consisting of clusters of contiguous CpG islands or gene families. Hypermethylation of various gene clusters has been reported in many types of cancer. In this study, we conducted methyl-binding domain capture (MBDCap) sequencing (MBD-seq) analysis on a breast cancer cohort consisting of 77 patients and 10 normal controls, as well as a panel of 38 breast cancer cell lines. RESULTS: Bioinformatics analysis determined seven gene clusters with a significant difference in overall survival (OS) and further revealed a distinct feature that the conservation of a large gene cluster (approximately 70 kb) metallothionein-1 (MT1) among 45 species is much lower than the average of all RefSeq genes. Furthermore, we found that DNA methylation is an important epigenetic regulator contributing to gene repression of MT1 gene cluster in both ERalpha positive (ERalpha+) and ERalpha negative (ERalpha-) breast tumors. In silico analysis revealed much lower gene expression of this cluster in The Cancer Genome Atlas (TCGA) cohort for ERalpha + tumors. To further investigate the role of estrogen, we conducted 17beta-estradiol (E2) and demethylating agent 5-aza-2'-deoxycytidine (DAC) treatment in various breast cancer cell types. Cell proliferation and invasion assays suggested MT1F and MT1M may play an anti-oncogenic role in breast cancer. CONCLUSIONS: Our data suggests that DNA methylation in large contiguous gene clusters can be potential prognostic markers of breast cancer. Further investigation of these clusters revealed that estrogen mediates epigenetic repression of MT1 cluster in ERalpha + breast cancer cell lines. In all, our studies identify thousands of breast tumor hypermethylated regions for the first time, in particular, discovering seven large contiguous hypermethylated gene clusters.

Jaiswal, P. S., et al. (2018). "Cyamopsis tetragonoloba type 1 metallothionein (CtMT1) gene is upregulated under drought stress and its protein product has an additional C-X-C motif and unique metal binding pattern." Int J Biol Macromol 119: 1324-1334.

Metallothioneins (MTs) are involved in cellular homeostasis of essential metal ions and detoxification of nonessential metal ions. We report here the identification of four MT genes, CtMT1, CtMT2, CtMT3 and CtMT4, encoding CtMT1, CtMT2, CtMT3 and CtMT4 proteins, respectively, from the industrial guar crop. The primary structures of last three proteins were similar to those of respective MT proteins of other plants but the CtMT1 protein primary structure was different from the other plant MT1

proteins in having an additional C-X-C motif. The four MT genes showed tissue specific expression patterns suggesting their specific roles in different tissues. High expression of CtMT1 gene was observed in roots and nodules whereas CtMT2 and CtMT3 genes showed high expression in leaves. The expression of CtMT4 gene was high in seeds. The qRT-PCR studies revealed upregulation in expression of CtMT1 gene under drought stress. Recombinant CtMT1 protein was produced in E. coli Rosetta cells and purified by metal affinity chromatography. The purified protein showed antioxidant property and the order of its metal ion binding affinities was Cu(2+)>Zn(2+)>Fe(2+)>Cd(2+). This information about CtMT1 protein is expected to be useful in understanding its role in drought tolerance and other physiological processes of guar.

Jiang, Y. and Y. J. Kang (2004). "Metallothionein gene therapy for chemical-induced liver fibrosis in mice." Mol Ther 10(6): 1130-1139.

Liver fibrogenesis resulting from a diversity of pathological changes involves a disturbance in mineral, in particular zinc, homeostasis. The present study was undertaken to determine whether gene therapy with metallothionein (MT), a small protein critically involved in the regulation of zinc homeostasis, can improve the recovery of liver fibrosis in a mouse model. Wild-type (WT) mice treated with carbon tetrachloride in corn oil twice a week at 1 ml/kg for 4 weeks developed a reversible liver fibrosis upon removal of the chemical, correlating with a high level of hepatic MT; but those treated for 8 weeks developed an irreversible liver fibrosis along with low levels of hepatic MT. The same carbon tetrachloride treatment for 4 weeks resulted in an irreversible liver fibrosis in MT-knockout (MT-KO) mice. Adenoviral delivery of the human MT-II gene (approved symbol MT2A) through intravenous injection reversed the fibrosis along with increased hepatocyte regeneration within 3 days in both WT and MT-KO mice with irreversible fibrosis. The MT elevation was associated with increased activities of collagenases in the liver. This study indicates that MT makes a critical contribution to the reversal of chemical-induced hepatic fibrosis and has therapeutic potential for patients with certain liver fibrosis.

Jin, S., et al. (2006). "A metallothionein-like protein of rice (rgMT) functions in E. coli and its gene expression is induced by abiotic stresses." Biotechnol Lett 28(21): 1749-1753.

A metallothionein-like (rgMT) gene was isolated from a rice (Oryza sativa L.) root cDNA library that was prepared from plants grown under NaHCO3 stress. The rgMT gene expression was induced in rice leaves and roots under several abiotic stresses from salts (NaCl and NaHCO3), drought (PEG) and metals (CuCl2, ZnCl2, CdCl2). The results suggested that the rgMT gene was expressed in response to environmental stresses. The rgMT gene was expressed in Escherichia coli, and the final yield of the purified rgMT protein was 4.8 mg g(-1) dry cells. Tolerance of E. coli expressing GST-rgMT fusion protein to Cu2+, Zn2+ and Cd2+ was enhanced, and cells dry weight increased 0.04 mg, 0.17 mg and 0.07 mg in 1 ml culture treated with either CuCl2, ZnCl2 or CdCl2, respectively, compared with control after 6 h culture.

Jin, S., et al. (2014). "Expression of the rgMT gene, encoding for a rice metallothionein-like protein in Saccharomyces cerevisiae and Arabidopsis thaliana." J Genet 93(3): 709-718.

Metallothioneins (MTs) are cysteine-rich proteins of low molecular weight with many attributed functions, such as providing protection against metal toxicity, being involved in regulation of metal ions uptake that can impact plant physiology and providing protection against oxidative stress. However, the precise function of the metallothionein-like proteins such as the one coded for rgMT gene isolated from rice (Oryza sativa L.) is not completely understood. The whole genome analysis of rice (O. sativa) showed that the rgMT gene is homologue to the Os11g47809 on chromosome 11 of O. sativa sp. japonica genome. This study used the rgMT coding sequence to create transgenic lines to investigate the subcellular localization of the protein, as well as the impact of gene expression in yeast (Saccharomyces cerevisiae) and Arabidopsis thaliana under heavy metal ion, salt and oxidative stresses. The results indicate that the rgMT gene was expressed in the cytoplasm of transgenic cells. Yeast cells transgenic for rgMT showed vigorous growth compared to the nontransgenic controls when exposed to 7 mM CuCl2, 10 mM FeCl2, 1 M NaCl, 24 mM NaHCO3 and 3.2 mM H2O2, but there was no significant difference for other stresses tested. Similarly, Arabidopsis transgenic for rgMT displayed significantly improved seed germination rates over that of the control when the seeds were stressed with 100 muM CuCl2 or 1 mM H2O2. Increased biomass was observed in the presence of 100 muM CuCl2, 220 muM FeCl2, 3 mM Na2CO3, 5 mM NaHCO3 or 1 mM H2O2. These results indicate that the expression of the rice rgMT gene in transgenic yeast and Arabidopsis is implicated in improving their tolerance for certain salt and peroxide stressors.

Jin, S., et al. (2017). "Functional characterization of a type 2 metallothionein gene, SsMT2, from alkaline-tolerant Suaeda salsa." Sci Rep 7(1): 17914.

A type 2 metallothionein gene, SsMT2, was cloned from Suaeda salsa, a salt- and alkali-tolerant plant, which is dominant species on the saline/alkali soil of northeast China. The SsMT2 gene was expressed in all organs except the flower and its

expression was induced by various stresses such as CdCl2, NaCl, NaHCO3, and H2O2 treatments. SsMT2-transgenic yeast (Saccharomyces cerevisiae) and plants (Arabidopsis thaliana) showed significantly increased resistance to metal, salt and oxidant stresses. These transgenics accumulated more Cd(2+), but less Na(+) than their wild type counterparts. SsMT2 transgenic Arabidopsis maintained lower level of H2O2 than wild type plants did in response to the stress treatments. These results demonstrated that the SsMT2 gene plays an important role in reactive oxygen species scavenging and confers enhanced metal and oxidant tolerance to plants.

Juang, H. H., et al. (2013). "Metallothionein 3: an androgen-upregulated gene enhances cell invasion and tumorigenesis of prostate carcinoma cells." Prostate 73(14): 1495-1506.

BACKGROUND: Metallothioneins (MT1, MT2, MT3, and MT4) are regarded as modulators regulating a number of biological processes including cell proliferation, differentiation, and invasion. We determined the effects of androgen, cadmium, and arsenic on MT1/2 and MT3 in prostate carcinoma cells, and evaluated the functional effects of MT3 on cell proliferation, invasion, and tumorigenesis. METHODS: We determined the expression of MT1/2 and MT3 in prostate carcinoma cells by immunoblotting assays or real-time reverse transcription-polymerase chain reactions. The effects of ectopic MT3 overexpression or MT3-knockdown on cell proliferation, invasion, and tumorigenesis were determined by (3) H-thymidine incorporation, matrigel invasion, and murine xenograft studies. The effects of androgen, cadmium, and arsenic on target genes were assessed using immunoblotting and reporter assays. RESULTS: Androgen, cadmium, and arsenic treatments enhanced gene expression of MT1/2 and MT3 in prostate carcinoma LNCaP cells. Results of immunohistochemical staining indicated MT3 overexpression was found predominantly in the nuclear areas of PC-3 cells overexpressing MT3. Overexpression of MT3 significantly increased cell proliferation, invasion, and tumorigenic activities in PC-3 cells in vitro and in vivo. MT3 overexpression downregulated the gene expressions of N-myc downstream regulated gene 1 (Ndrg1) and maspin, and attenuated blocking effects of doxorubicin in PC-3 cells on cell proliferation. MT3-knockdown enhanced Ndrg1 and maspin expressions in LNCaP cells. CONCLUSIONS: The experiments indicate that MT3 is an androgen-upregulated gene, and promotes tumorigenesis of prostate carcinoma cells. The downregulation of Ndrg1 and maspin gene expressions appears to account for the enhancement of proliferative and invasive functions of MT3 in PC-3 cells.

Kadota, Y., et al. (2010). "Enhanced metallothionein gene expression induced by mitochondrial oxidative stress is reduced in phospholipid hydroperoxide glutathione peroxidase-overexpressed cells." Eur J Pharmacol 626(2-3): 166-170.

Mitochondria are major compartments in cells responsible for generating reactive oxygen species, which can cause the development of diabetes, Parkinson's disease and premature aging. Antioxidant systems in mitochondria are important for the prevention of diseases and reduction in the speed of aging. We investigated whether the reactive oxygen species generated in mitochondria induced the expression of metallothionein as an antioxidant. We compared the expression level of metallothionein mRNA in mitochondrial phospholipid hydroperoxide glutathione peroxidase (PHGPx)-overexpressed (PHGPx-ov) cells with that in control cells. These cells were treated with respiratory inhibitors, including rotenone and 2, 4-dinitrophenol; under these conditions, the PHGPx-ov cells were more resistant to cell death than the control cells. In addition, the intracellular reactive oxygen species level that was induced by these inhibitors was lower in PHGPx-ov cells than in control cells. This indicates that PHGPx degrades the membrane phospholipid hydroperoxide that is formed via the reactive oxygen species generated in mitochondria. The enhanced expression of metallothionein-I and metallothionein-II mRNA in rotenone-treated control cells was significantly decreased in rotenone-treated PHGPx-ov cells, suggesting that the hydrogen peroxide that is formed by superoxide anions generated in mitochondria diffuse into the cytosol and induce metallothionein mRNA expression. Conversely, the expression of manganese-superoxide dismutase (Mn-SOD) mRNA, which is localized in mitochondria, was not correlated with the intracellular reactive oxygen species level that was induced by rotenone treatment. These results suggest that metallothionein expression is sensitively and strictly regulated by the oxidative state that is induced by mitochondrial respiration.

Kaewprasert, S., et al. (2000). "Dietary beta- and gamma-cyclodextrins stimulation of hepatic metallothionein gene expression in rats." Biosci Biotechnol Biochem 64(11): 2469-2473.

This study investigated whether hepatic metallothionein gene expression is affected by dietary cyclodextrins. Young male Wistar rats were fed a basal diet or cyclodextrin-supplemented (50 g of cyclodextrin per kg diet) diets for 7 d. Copper content in the liver did not show any significant changes among rats fed the basal, beta- and gamma-cyclodextrin diets. There were no differences in liver or serum zinc among groups. Copper content in serum was markedly decreased in rats fed the gamma-cyclodextrin-supplemented diet. Liver metallothionein mRNA levels were significantly elevated in both beta-

and gamma-cyclodextrins-fed rats, but not in alpha-cyclodextrin-fed rats. Thus, the increase in hepatic metallothionein mRNA levels might be due to this mechanism except for the contents of copper and zinc in the liver.

Kaina, B., et al. (1990). "Overexpressed human metallothionein IIA gene protects Chinese hamster ovary cells from killing by alkylating agents." Proc Natl Acad Sci U S A 87(7): 2710-2714.

Experiments were designed to detect survival advantages that cells gain by overexpressing metallothionein (MT). Chinese hamster ovary K1-2 cells and an x-ray-sensitive derivative were transfected with a bovine papillomavirus (BPV)-linked construct carrying the human metallothionein IIA (hMT-IIA) gene. Transfectants survived 40-fold higher levels of cadmium chloride, harbored at least 30 copies of hMT-IIA, and contained 25- to 166-fold more MT than the parent cells. Even under conditions of reduced glutathione synthesis, the transfectants were not more resistant to the lethal effects of ionizing radiation and bleomycin than the parent cells. Thus free radicals generated by these agents cannot be scavenged efficiently by MT in vivo. The hMT-IIA transfectants, however, but not control transfectants harboring a BPV-MT promoter-neo construct, tolerated significantly higher doses of the alkylating agents N-methyl-N-nitrosourea and N-methyl-N'-nitro-N-nitrosoguanidine. Resistance and MT overexpression occurred irrespective of selection and cultivation in cadmium and zinc. There was no increase in resistance to methyl methanesulfonate and N-hydroxyethyl-N-chloroethylnitrosourea. MT did not affect the degree of overall DNA methylation after N-methyl-N-nitrosourea treatment nor the level of O6-methylguanine-DNA methyltransferase. The results suggest that MT participates as a cofactor or regulatory element in repair or tolerance of toxic alkylation lesions.

Kamaladini, H., et al. (2011). "Metal inducible activity of the oil palm metallothionein-like gene promoter (MT3-A) in prokaryotes." J Biosci Bioeng 111(2): 217-225.

Reporter gene activity under the regulation of the oil palm metallothionein-like gene, MT3-A promoter was assessed in prokaryotes. Vector constructs containing MT3-A promoter with (W1MT3-A) and without (W2MT3-A) five prime untranslated region (5'-UTR) fused to ss-glucuronidase (GUS) gene in pCAMBIA 1304 vector were produced. 5'-rapid amplification of cDNA ends (RACE) using mRNA isolated from Escherichia coli and Agrobacterium tumefaciens harboring W1MT3-A confirmed that fusion transcripts of MT3-A 5'-UTR-GUS were successfully produced in both bacteria. Competitive PCR and GUS fluorometric assay showed changes in the level of GUS gene transcripts and enzyme activity in response to increasing concentrations of Cu(2)+ and Zn(2)+. The application of Cu(2)+ increased GUS activity and GUS mRNA level in both bacteria. In E. coli, a high level of GUS activity driven by W1MT3-A and W2MT3-A was observed in treatment with 25 muM Cu(2)+ resulting in an increase in the GUS mRNA level to 7.2 and 7.5 x 10(-)(4) pmol/mul respectively, compared to the control (5.1 x 10(-)(4) pmol/mul). The lowest GUS activity and GUS mRNA level were obtained for W1MT3-A and W2MT3-A in the presence of 100 muM Cu(2)+ in both bacteria compared to the control (without Cu(2)+). The application of different Zn(2)+ concentrations resulted in a strong decrease in the GUS activity and GUS mRNA level in E. coli and A. tumefaciens. These findings showed that the oil palm MT3-A promoter is functional in prokaryotes and produced detectable GUS transcripts and enzyme activities. This promoter may potentially be used in prokaryotic systems which require metal inducible gene expression.

Kamaladini, H., et al. (2013). "Breaking-off tissue specific activity of the oil palm metallothionein-like gene promoter in T(1) seedlings of tomato exposed to metal ions." J Plant Physiol 170(3): 346-354.

Metallothioneins (MTs) are cysteine-rich metal-binding proteins that are involved in cell growth regulation, transportation of metal ions and detoxification of heavy metals. A mesocarp-specific metallothionein-like gene (MT3-A) promoter was isolated from the oil palm (Elaeis guineensis Jacq). A vector construct containing the MT3-A promoter fused to the beta-glucuronidase (GUS) gene in the pCAMBIA 1304 vector was produced and used in Agrobacterium-mediated transformation of tomato. Histochemical GUS assay of different tissues of transgenic tomato showed that the MT3-A promoter only drove GUS expression in the reproductive tissues and organs, including the anther, fruit and seed coat. Competitive RT-PCR and GUS fluorometric assay showed changes in the level of GUS mRNA and enzyme activity in the transgenic tomato (T(0)). No GUS mRNA was found in roots and leaves of transgenic tomato. In contrast, the leaves of transgenic tomato seedlings (T(1)) produced the highest GUS activity when treated with 150 muM Cu(2+) compared to the control (without Cu(2+)). However, Zn(2+) and Fe(2+) treatments did not show GUS expression in the leaves of the transgenic tomato seedlings. Interestingly, the results showed a breaking-off tissue-specific activity of the oil palm MT3-A promoter in T(1) seedlings of tomato when subjected to Cu(2+) ions.

Kambadur, R., et al. (1990). "Cloned yeast and mammalian transcription factor TFIID gene products support basal but not activated metallothionein gene transcription." Proc Natl Acad Sci U S A 87(23): 9168-9172.

Transcription factor IID (TFIID), the "TATA binding factor," is thought to play a key role in the regulation of eukaryotic transcriptional initiation. We have studied the role of TFIID in the transcription of the yeast metallothionein gene, which is regulated by the copper-dependent activator protein ACE1. Both basal and induced transcription of the metallothionein gene require TFIID and a functional TATA binding site. Crude human and mouse TFIID fractions, prepared from mammalian cells, respond to stimulation by ACE1. In contrast, human and yeast TFIID proteins expressed from the cloned genes do not respond to ACE1, except in the presence of wheat germ or yeast total cell extracts. These results indicate that the cloned TFIID gene products lack a component(s) or modification(s) that is required for regulated as compared to basal transcription.

Kan, G., et al. (2019). "Cloning and functional characterization of a novel metallothionein gene in Antarctic sea-ice yeast (Rhodotorula mucilaginosa)." J Basic Microbiol 59(9): 879-889.

Metallothionein (MT) is a low-molecular-weight protein with a high metal binding capacity and plays a key role in organism adaptation to heavy metals. In this study, a metallothionein gene was successfully cloned and sequenced from Antarctic sea-ice yeast Rhodotorula mucilaginosa AN5. Nucleotide sequencing and analysis revealed that the gene had four exons interrupted by three introns. MTs complementary DNA (named as RmMT) had an open reading frame of 321 bp encoding a 106 amino acid protein with a predicted molecular weight of 10.3 kDa and pI of 8.49. The number of amino acids and distribution of cysteine residues indicated that RmMT was a novel family of fungal MTs. Quantitative real-time polymerase chain reaction analysis showed that RmMT expression was elevated under copper-induced stress. The RmMT gene was transferred into E. coli and the RmMT expressing bacteria showed improved tolerance to copper ion and increased accumulation of heavy metals, such as Cu(2+) , Pb(2+) , Zn(2+) , Cd(2+) , and Ag(+) . Moreover, in vitro studies, purified recombinant RmMT demonstrated that it could be used as a good scavenger of superoxide anion, hydroxyl, and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicals. In summary, these results demonstrate that RmMT plays a key role in the tolerance and bioaccumulation of heavy metals.

Kanda, M., et al. (2009). "Detection of metallothionein 1G as a methylated tumor suppressor gene in human hepatocellular carcinoma using a novel method of double combination array analysis." Int J Oncol 35(3): 477-483.

Gene expression profiling or karyotyping analysis has made it possible to identify novel genes with altered expressions or copy numbers that have not been previously reported in liver cancer. On the same HCC sample, we performed double array analysis, both expression profiling and karyotyping analysis using a single nucleotide polymorphism (SNP) array in an attempt to find a novel tumor suppressor gene for its prognostic marker. We conducted expression array and SNP chip array using tumor and corresponding non-tumor tissues from the resected liver specimen of a 68-year-old woman who had chronic hepatitis type C. Additionally, we performed quantitative real-time reverse transcription polymerase chain reaction (PCR) and methylation-specific PCR (MSP) for gene detection using specimens from 48 patients with HCC, and investigated their correlation with the prognosis. Metallothionein (MT) 1G gene located on 16q13 showed a decreased expression in tumor tissue. The copy number by SNP chip array revealed no loss of heterozygosity since no deletions were detected in 16q13, and HCC tissue showed AB call in both SNPs next to MT1G. In quantitative real-time PCR using 48 HCC clinical samples, mRNA expression of MT1G decreased significantly compared with that in corresponding non-cancerous liver tissues ($p<0.0323$). Twenty-nine (60.4%) of 48 HCCs gave a positive result in MSP, indicating a poorer prognosis than the negative group, although the difference was not significant ($p<0.0978$). Our results indicated that MT1G acts as a tumor suppressor gene in HCC. Moreover, findings suggested that the mechanisms of MT1G silencing are related to promoter hypermethylation.

Kanekiyo, M., et al. (2002). "Metallothionein is required for zinc-induced expression of the macrophage colony stimulating factor gene." J Cell Biochem 86(1): 145-153.

Macrophage colony stimulating factor (M-CSF) plays an important role in the proliferation and differentiation of mononuclear phagocytes. The present study investigates the effect of zinc on M-CSF expression in MC3T3-E1 and L929 cells. Zinc dose-dependently increased M-CSF mRNA levels. The time-course of zinc-induced M-CSF mRNA expression peaked at 6 h. Stability studies of mRNA using actinomycin D revealed that zinc does not affect M-CSF mRNA stability. We examined the function of the M-CSF gene promoter using a luciferase reporter assay. A construct containing the -467/+39 region of the promoter was upregulated by zinc. In the presence of cycloheximide, zinc did not induce a greater increase in the M-CSF mRNA than cycloheximide alone. To confirm the effect of MT on M-CSF mRNA expression, mouse lung fibroblasts (MLFs) were prepared from MT+/+ and MT-/- mice. Zinc induced an increase in the expression of M-CSF in MT+/+ MLFs, but this response was not evident in MT-/- MLFs. Moreover, overexpression of MT upregulated M-CSF mRNA expression as well as M-CSF secretion. Our findings

suggest that MT expression mediates zinc regulation of M-CSF gene expression at the transcriptional level.

Karasawa, M., et al. (1987). "Regulation of metallothionein gene expression by 1 alpha,25-dihydroxyvitamin D3 in cultured cells and in mice." Proc Natl Acad Sci U S A 84(24): 8810-8813.

1 alpha,25-Dihydroxyvitamin D3 [1 alpha,25(OH)2D3], a hormonally active form of vitamin D3, has been shown to modulate cell differentiation and tumor promotion. This report demonstrates that mRNA of the metallothionein (MT) gene was induced by 1 alpha,25(OH)2D3 in cultured epidermal keratinocytes and also in liver, kidney, and skin tissues when 1 alpha-hydroxyvitamin D3, a synthetic precursor of 1 alpha,25(OH)2D3, was applied in vivo. Exposure of FRSK cells, a cell line derived from fetal rat skin keratinocytes, to 1 alpha,25(OH)2D3 at 5 ng/ml (12 nM) increased MT mRNA to almost the same extent as that induced by 10 microM dexamethasone or 1 microM CdCl2. This increase in the level of MT mRNA was evident within 2 hr and was maximal 12-24 hr after the addition of 1 alpha,25(OH)2D3. The induction was dose-dependent with concentrations of 1 alpha,25(OH)2D3 from 0.05 to 5.0 ng/ml. Amounts of MT increased with the increase of MT mRNA induced by 1 alpha,25(OH)2D3. Of the derivatives of vitamin D3 tested, only 1 alpha,25(OH)2D3 caused marked induction. Treatment with cycloheximide did not inhibit MT mRNA induction by 1 alpha,25(OH)2D3. 1 alpha,25(OH)2D3 induced MT mRNA in primary cultures of mouse epidermal keratinocytes but not in IAR-20, a liver cell line. 1 alpha,25(OH)2D3 had a similar effect in vivo: oral administration of 1 alpha-hydroxyvitamin D3 to mice resulted in increased levels of MT mRNA in the liver, kidney, and skin 24 hr later. Increase in the level of MT mRNA may be relevant to some biological actions of 1 alpha,25(OH)2D3.

Karin, M., et al. (1983). "Expression and regulation of a human metallothionein gene carried on an autonomously replicating shuttle vector." Proc Natl Acad Sci U S A 80(13): 4040-4044.

A human metallothionein (MT) gene was inserted into a bovine papillomavirus (BPV) vector. The chimeric vector (pMTII-BPV) transforms rodent fibroblasts to a cadmium-resistant phenotype. The resistance is due to the high level of expression of human MT-II in those cells. The vector is maintained in the cells as a free replicating plasmid, present at about 10--15 copies per cell. Transcription of the episomal human MT-IIA gene is initiated from its authentic start sites and is regulated by the level of cadmium in the growth medium. The presence of the human MT-IIA gene allows the BPV replicon to function even though it is ligated to an intact copy of pBR322. Due to the presence of plasmid origins of replication and dominantly acting selective markers functional in both Escherichia coli and mammalian cells, pMTII-BPV can be used as a shuttle vector.

Karin, M., et al. (1984). "Activation of a heterologous promoter in response to dexamethasone and cadmium by metallothionein gene 5'-flanking DNA." Cell 36(2): 371-379.

Human metallothionein-IIA (hMT-IIA) gene expression is regulated by heavy metals and glucocorticoids. When the cloned hMT-IIA gene or its 5'-flanking DNA structure fused to herpes simplex virus thymidine kinase (HSV-TK) structural gene sequences were transferred into TK- Rat 2 fibroblasts, both genes were inducible by Cd++ and/or dexamethasone. Placement of the hMT-IIA gene 5'-flanking region, either intact of deleted in its TATA box and cap site, upstream of the HSV-TK gene promoter rendered the latter both glucocorticoid- and heavy metal-inducible. Thus the structure that mediates both Cd++ and glucocorticoid responsiveness is present in the hMT-IIA gene 5'-flanking DNA, does not require its TATA box or cap site, and can activate a heterologous promoter.

Karin, M., et al. (1984). "Characterization of DNA sequences through which cadmium and glucocorticoid hormones induce human metallothionein-IIA gene." Nature 308(5959): 513-519.

Deletion experiments have defined two stretches of DNA (genetic elements), lying close to the promoter for a human gene for metallothionein, that separately mediate the induction of the gene by heavy metal ions, particularly cadmium, and by glucocorticoid hormones. The element responsible for induction by cadmium is duplicated, yet a single copy is fully functional; the element responsible for induction by glucocorticoid hormones is coincident with the DNA-binding site for the glucocorticoid hormone receptor.

Karin, M., et al. (1985). "Interleukin 1 regulates human metallothionein gene expression." Mol Cell Biol 5(10): 2866-2869.

Incubation of cultured human cells with interleukin 1 leads to increased expression of the human metallothionein-IIA gene. Recently, metallothionein has been shown to be an efficient free radical scavenger, and induction by interleukin 1 may be part of a protective response to minimize damage by hydroxyl radicals.

Karin, M. and R. I. Richards (1982). "Human metallothionein genes--primary structure of the metallothionein-II gene and a related processed gene." Nature 299(5886): 797-802.

The complete nucleotide sequence of two of the human metallothionein gene family has been compared. One is a functional metallothionein-II gene, the other a pseudogene, lacking introns, terminating in

a poly(A) tail and flanked by two direct repeats. In addition, we have detected a size polymorphism associated with the processed gene in the population, examined, and we have observed a region of apparent secondary structure homology between of 5' flanking region of the functional metallothionein-II gene and that of a mouse metallothionein-I gene.

Karin, M. and R. I. Richards (1984). "The human metallothionein gene family: structure and expression." Environ Health Perspect 54: 111-115.

Metallothioneins (MTs) are encoded by a multigene family in man. We have isolated those genes and analyzed the structure of some of them. The MT-II variant is encoded by a single functional gene: MT-IIA. The MT-IIB gene is a processed pseudogene derived from a reverse transcript of MT-II mRNA. On the other hand, the MT-I class of variants are encoded by a large number of genes, arranged in tandem. The MT-IIA and the MT-IA genes show a differential response to glucocorticoid hormones and heavy metals, yet they are both expressed in primary human fibroblasts and in HeLa cells. Expression of both of those genes, in high level after transfer on bovine papilloma virus vectors, leads to increased resistance of the host cells to cadmium-induced toxicity.

Kasutani, K., et al. (1998). "Requirement for cooperative interaction of interleukin-6 responsive element type 2 and glucocorticoid responsive element in the synergistic activation of mouse metallothionein-I gene by interleukin-6 and glucocorticoid." Toxicol Appl Pharmacol 151(1): 143-151.

Metallothionein (MT)-inducing activity of interleukin (IL)-6 depends on the presence of glucocorticoid in hepatic cells. The synergistic action of IL-6 and glucocorticoid was observed in the transcriptional activation of the mouse MT (mMT)-I gene. We found that a 281-bp promoter was sufficient for IL-6 and glucocorticoid stimulation. Our inspection of this region revealed the putative type 1 and 2 IL-6 responsive elements (REs). Functional analyses of these regions were performed using luciferase reporter constructs, and it was observed that the type 2 IL-6RE exerted the major response to the IL-6 signal. The transcriptional factor binding to type 1 IL-6RE, nuclear factor-IL-6, hardly contributed to the activation of the mMT-I promoter by IL-6 and glucocorticoid. A glucocorticoid responsive element (GRE) was also required for the synergistic activation by IL-6 and glucocorticoid. Interestingly, this synergism was not observed when the type 2 IL-6RE and the GRE were kept apart. Therefore, the synergistic activation of the mMT-I gene by IL-6 and glucocorticoid may require not only that signal transducers and activators 3 (Stat3) and the glucocorticoid receptor (GR) bind to their respective responsive elements, but also that Stat3 and the GR physically interact with one another.

Katakai, K., et al. (2001). "Nitric oxide induces metallothionein (MT) gene expression apparently by displacing zinc bound to MT." Toxicol Lett 119(2): 103-108.

The metal binding protein metallothionein (MT) is involved in zinc homeostasis since it typically binds large amounts of zinc. Free zinc can control MT gene expression by interacting with metal-sensitive transcription factors. However, the precise factors governing intracellular release of metal ions from MT remain unknown. Aerobic nitric oxide (NO) can nitrosate thiol groups in proteins, and MT-bound cadmium is released by NO exposure. Thus, we hypothesized that NO may also be effective at displacing zinc from MT in cultured cells and that this could be an important physiological control mechanism in zinc homeostasis and utilization. In this study, DETA/NO, an agent that spontaneously generates NO with a 20-h half life in physiological media, was used to study the release of zinc from MT and the induction of MT in TRL1215 cells (a normal rat liver cell line). Zinc or cadmium was given at levels inducing MT production, followed by DETA/NO (20-200 microM) to produce controlled NO exposure in both cell lines. Although both metals activated MT gene expression, MT-I mRNA and MT protein were further increased when DETA/NO was given after zinc or cadmium treatment. Additionally, NO from DETA/NO clearly displaced MT-bound zinc, as evidenced by G-75 gel-filtration chromatography. The released zinc or cadmium probably then stimulates further MT gene expression. These results suggest that NO may play an important role in regulation of cellular zinc homeostasis by providing a controlled release mechanism for metal ions stored in MT, and NO-mediated release of MT-bound zinc could in turn activate gene expression, such as with the MT gene.

Kay, J., et al. (1987). "Metallothionein gene expression and cadmium toxicity in freshwater fish." Experientia Suppl 52: 627-630.

Certain species of fish e.g. rainbow trout, are particularly susceptible to poisoning by cadmium in their aquatic environment whereas others e.g. roach and stone loach, are much more resistant. It is postulated that the vulnerability of the salmonids arises because 1) existing metallothionein (MT) in the tissues of these fish is unable to bind cadmium and 2) the toxic metal (in contrast to zinc) cannot switch on the gene(s) for apo-thionein production de novo. Consequently, cadmium is sequestered in the liver, kidney and gills of these fish by two low mol.wt. non-metallothionein proteins for which no excretion mechanism appears to exist.

Kayaalti, Z., et al. (2011). "Distributions of interleukin-6 (IL-6) promoter and metallothionein 2A (MT2A) core promoter region gene polymorphisms and their

associations with aging in Turkish population." Arch Gerontol Geriatr 53(3): 354-358.

Aging is determined as the product of an interaction among genetic, environmental and lifestyle factors. As interleukin-6 (IL-6) and metallothioneins (MTs) are related to inflammation and oxidative stress response, their genes are appropriate candidate for aging, age related diseases and infections. The aim of this study was to investigate the association between the IL-6 -174 G/C promoter region and MT2A -5 A/G core promoter region single nucleotide polymorphisms (SNPs) with longevity in Turkish population. Blood samples were collected from 354 individuals between 18 and 95 years of age. Individuals were classified into four groups according to their ages as 20-40, 41-60, 61-80, >80. IL-6 and MT2A polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Mean ages of individuals with IL-6 -74 C- carriers and C+ carriers were 49.82+/-20.45 years and 59.82+/-16.82 years, respectively. For the MT2A polymorphism, mean ages were estimated as 56.18+/-19.50 years for G- carriers and 47.59+/-13.45 years for G+ carriers. As a result, when the IL-6 and MT2A polymorphisms were compared with the mean ages and age groups, statistically significant associations were found ($p<0.001$ and $p<0.05$, respectively). In conclusion, these data support that the IL-6 -174 C+ carriers and MT2A -5 G- carriers may be more advantageous for longevity.

Kheradmand, F., et al. (2010). "Differential gene-expression of metallothionein 1M and 1G in response to zinc in sertoli TM4 cells." Iran Biomed J 14(1-2): 9-15.

BACKGROUND: Zinc (Zn) as an important trace element is essential for testicular development and spermatogenesis. Molecular mechanism of Zn action in the reproductive system may be related to metal binding low-molecular weight proteins, metallothioneins (MT). Our objective was to determine the effect of Zn on two important isoforms of MT, MT1M and MT1G genes expression on testicular sertoli cells. METHODS: Cultured sertoli TM4 cells were exposed to different concentrations of Zn at different time points. Cellular uptake of Zn was tested using flame atomic absorption spectrometry. The cellular viability and gene expression were assessed by MTT and real-time PCR methods, respectively. RESULTS: The treated cells resulted in higher Zn concentration and cellular viability. The expression of MT1M and MT1G genes in the treated cells were greater than those of the untreated cells (P less than 0.05). In the high dosage treated group (100 and 500 muM), Zn concentration and expression of MT1M and MT1G genes increased three h after treatment; MT1G gene expression increased more at sixth h. At 18th h of treatment, the expression of both genes especially MT1G, increased dramatically while Zn concentration decreased. CONCLUSION: Since the increase of MT1G mRNA was coincident with cellular Zn level, it seems that MT1G has a more prominent role than MT1M in the homeostasis of Zn. In addition, Zn at dosage of 50 muM (pharmacologic concentration) may protect cells by increasing the expression of MT genes at longer periods.

Kille, P., et al. (1990). "The expression of a synthetic rainbow trout metallothionein gene in E. coli." Biochim Biophys Acta 1048(2-3): 178-186.

A synthetic gene for rainbow trout metallothionein was constructed and inserted into a dual origin plasmid where expression was induced by a temperature shift in a proteinase-deficient strain of Escherichia coli. The recombinant protein was purified to homogeneity, and a partial amino acid sequence was determined to confirm its identity. Its immunochemical characteristics were similar to those of native metallothionein from rainbow trout. The amounts of recombinant metallothionein produced were quantified in soluble cell extracts by ELISA. Low concentrations were detected when growth was performed either in L-broth or defined (GMM-II) medium. Supplementation of the medium with zinc or copper had no effect on the amount of metallothionein produced. By contrast, when cadmium was included in either L-broth or GMM-II medium, much higher concentrations of the protein within the cells (approx. 13 micrograms/mg soluble cell protein) were detected. This stabilisation of the protein by metal reconstitution in vivo is considered in relation to the selective uptake/exclusion of metals by the cells and its significance for the scavenging of certain precious or toxic heavy metals is discussed.

Kim, J. H. and J. C. Kang (2016). "The immune responses and expression of metallothionein (MT) gene and heat shock protein 70 (HSP 70) in juvenile rockfish, Sebastes schlegelii, exposed to waterborne arsenic (As(3+))." Environ Toxicol Pharmacol 47: 136-141.

Juvenile rockfish, Sebastes schlegelii (mean length 16.4+/-1.9cm, and mean weight 71.6+/-6.4g) were exposed for 20days with the different levels of waterborne arsenic concentration (0, 50, 100, 200 and 400mug/L). The plasma cortisol of S. schlegelii was significantly increased by the waterborne arsenit exposure. In the immune responses, the immunoglobulin M (Ig M) and lysozyme activity of S. schlegelii were significantly increased by the waterborne arsenic exposure. The acetylcholinesterase (AChE) activity of S. schlegelii was inhibited by the waterborne arsenic exposure. The substantial increases in the gene expression such as metallothionein (MT) and heat shock protein 70 (HSP 70) were observed by the waterborne arsenic exposure. The results demonstrated that waterborne arsenic exposure can

induce the significant alterations in the immune responses and specific gene expression of S. schlegelii.

Kim, J. H. and J. C. Kang (2016). "Oxidative stress, neurotoxicity, and metallothionein (MT) gene expression in juvenile rock fish Sebastes schlegelii under the different levels of dietary chromium (Cr(6+)) exposure." Ecotoxicol Environ Saf 125: 78-84.

Juvenile Sebastes schlegelii were exposed for 4 weeks with the different levels of dietary chromium (Cr(6+)) concentration (0, 30, 60, 120 and 200mg/kg). The superoxide dismutase (SOD) activity, glutathione S-transferase (GST) activity, and glutathione (GSH) level of liver and gill were evaluated after 4 weeks exposure. The SOD and GST activity of liver and gill was significantly increased in the concentration of 240mg/kg after 2 weeks and over 120mg/kg after 4 weeks, whereas a considerable decrease in the concentration of 240mg/kg after 2 weeks and over 120mg/kg after 4 weeks was observed in the GSH levels of liver and gill. In neurotoxicity, AChE activity was significatly inhibited in brain in the concentration of 240mg/kg after 2 weeks and over 60mg/kg after 4 weeks and muscle in the concentration of 240mg/kg after 2 weeks and over 120mg/kg after 4 weeks. Metallothionein (MT) gene in liver was considerably increased over 120mg/kg after 2 weeks and at 30, 120, and 240mg/kg after 4 weeks by dietary chromium exposure. The results indicate that dietary Cr exposure over 120mg/kg can induce substantial alterations in antioxidant responses, AChE activity and MT gene expression.

Kim, J. H., et al. (2017). "Antioxidant Responses, Neurotoxicity, and Metallothionein Gene Expression in Juvenile Korean Rockfish Sebastes schlegelii under Dietary Lead Exposure." J Aquat Anim Health 29(2): 112-119.

This study was conducted to assess toxic effects of dietary lead (Pb) exposure on Korean Rockfish Sebastes schlegelii. Juvenile rockfish were used to evaluate the oxidative stress, neurotoxicity, and metallothionein (MT) gene expression after dietary exposure to lead (as Pb(2+); 0, 30, 60, 120 and 240 mg/kg). Superoxide dismutase (SOD) activity, a measure of oxidative stress, was substantially elevated in the livers and gills of fish given dietary Pb greater than 60 mg/kg. Glutathione S-transferase (GST) activity in the liver and gill was significantly increased by dietary Pb > 60 mg/kg. A significant decrease in glutathione (GSH) level was observed in fish liver after exposure to dietary Pb > 30 mg/kg and in the gill after treatment with dietary Pb > 120 mg/kg. Acecyltholinesterase (AChE) was noticeably decreased in the brain by dietary Pb > 120 mg/kg and in the muscle by dietary Pb > 60 mg/kg. Metallothionein gene expression in the liver was stimulated significantly by the Pb exposure. Because dietary Pb exposure had a toxic effect on antioxidant responses, a neurotransmitter, and a specific immune expression in rockfish, the results of this study can be used to determine potential useful markers of Pb toxicity.

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Kimura, T., et al. (2008). "Chromium(VI) inhibits mouse metallothionein-I gene transcription by preventing the zinc-dependent formation of an MTF-1-p300 complex." Biochem J 415(3): 477-482.

Mouse MT-I (metallothionein-I) transcription is regulated by MTF-1 (metal-response-element-binding transcription factor-1) which is recruited to the promoter in response to zinc. Cr(VI) [chromium(VI)] pretreatment blocks zinc-activation of the endogenous MT-I gene and attenuates zinc-activation of MT-I-promoter-driven luciferase reporter genes in transient transfection assays. Chromatin immunoprecipitation assays revealed that Cr(VI) only modestly reduces recruitment of MTF-1 to the MT-I promoter in response to zinc, but drastically reduces the recruitment of RNA polymerase II. These results suggest that Cr(VI) inhibits the ability of MTF-1 to transactivate this gene in response to zinc. Zinc has recently been shown to induce the formation of a co-activator complex containing MTF-1 and the histone acetyltransferase p300 which plays an essential role in the activation of MT-I transcription. In the present study, co-immunoprecipitation assays demonstrated that Cr(VI) pretreatment blocks the zinc-induced formation of this co-activator complex. Thus Cr(VI) inhibits mouse MT-I gene expression in response to zinc by interfering with the ability of MTF-1 to form a co-activator complex containing p300 and recruiting RNA polymerase II to the promoter.

Kimura, T., et al. (2011). "Chromium (VI) inhibits mouse metallothionein-I gene transcription by modifying the transcription potential of the co-activator p300." J Toxicol Sci 36(2): 173-180.

The production of the heavy metal-binding proteins, the metallothioneins (MTs), is induced by heavy metals such as Zn, Cd, and Hg. MTs maintain Zn homeostasis and attenuate heavy metal-induced cytotoxicity by sequestering these metals and lowering their intracellular concentrations. Previously, we had reported that Zn induced the formation of a co-activator complex containing metal response element-binding transcription factor-1 (MTF-1) and the histone acetyltransferase (HAT), p300, which plays an essential role in the activation of MT-1 transcription. In addition, we had shown that Cr(VI) inhibits Zn-induced MT-1 transcription by preventing the Zn-dependent formation of the MTF-1-p300 complex. In the current study, we have shown that the inhibition by Cr(VI) was partially overcome by the overexpression of p300 or MTF-1 in an MT-I promoter-driven luciferase reporter assay system and have used real-time RT-PCR to determine

MT-I mRNA levels. It has been reported that Cr(VI) inhibits CYP1A1 transcription by crosslinking histone deacetylase (HDAC) to the promoter. The crosslink inhibits the recruitment of p300 to the MT-1 promoter and blocks HAT-dependent transactivation by p300. However, our results demonstrate that trichostatin A, an HDAC inhibitor, could not block the inhibitory effects of Cr(VI) on MT-1 transcription and that there were no significant differences in the in vitro inhibitory effects of Cr(VI), Cr(III), and Zn on p300 HAT activity. This suggests that the inhibitory effects of Cr(VI) on MT-I transcription may be due to its effects on the HAT-independent transactivation ability rather than the HAT-dependent, HDAC release-related transactivation ability of p300.

Kimura, T., et al. (2012). "Bis(L-cysteinato)zincate(lI) as a coordination compound that induces metallothionein gene transcription without inducing cell-stress-related gene transcription." J Inorg Biochem 117: 140-146.

Zinc is an essential micronutrient, deficiency of which results in growth retardation, immunodeficiency, and neurological diseases such as dysgeusia. Several zinc coordination compounds are used for zinc supplementation; however, supplemented zinc ions have no specificity and interact with various groups of molecules. Here, we found that, from a library of 30 zinc coordination compounds, bis(L-cysteinato)zincate(II), designated Z01, functioned as a metallothionein (MT) inducer. Z01 induced MT expression mediated by the transcription factor MTF-1, without inducing cell-stress-related heme oxygenase-1 gene expression at specific concentration. The zinc ion was necessary for the MT induction. (65)Zn incorporation following treatment with (65)Zn-labeled Z01 suggested that Z01 did not act as zinc ionophore despite its hydrophilicity. Electrophoretic mobility shift assays revealed that Z01 facilitates MTF-1-MRE complex formation, and, by inference, transfer of zinc from Z01 to MTF-1. Phosphorylated ERK levels were increased by ZnSO(4) treatment but not by Z01. Although our data do not definitely prove that Z01 is an MTF-1-specific activator, our observations suggest that zinc coordination compounds can regulate zinc distribution and act as zinc donors for specific molecules.

Kisa, D., et al. (2016). "Gene expression analysis of metallothionein and mineral elements uptake in tomato (Solanum lycopersicum) exposed to cadmium." J Plant Res 129(5): 989-995.

Heavy metals such as Cd are considered to be the most important pollutants in soil contamination. Cd is a non-essential element adversely affecting plant growth and development, and it has caused some physiological and molecular changes. Metallothioneins (MTs) are low molecular weight, cysteine-rich, and metal binding proteins. In this study, we aimed to evaluate the MT gene expression levels and minerals uptake in the tissues of Solanum lycopersicum exposed to Cd. The transcriptional expression of the MT genes was determined by real-time quantitative PCR. The MT genes were regulated by the Cd and the mineral elements uptake changed tissue type and applied doses. The MT1 and MT2 transcript levels increased in the roots, the leaves and the fruits of the tomato. The MT3 and MT4 transcript pattern changed according to the tissue types. The Cd treatment on the growth medium increased the Mg, Ca, and Fe content in both the leaves and fruits of the tomato. However, the Cd affected the mineral levels in the roots depending on the mineral types and doses. Also, the Cd content increased in the roots, the leaves, and the fruits of the tomato, respectively. The results presented in this study show that Cd has synergistic and/or antagonistic effects on minerals depending on the tissue types. These results indicate that the MT1 and MT2 expression pattern increased together with the Mg, Ca, and Fe content in both the leaves and the fruits of the tomato.

Kita, K., et al. (2006). "Potential effect on cellular response to cadmium of a single-nucleotide A --> G polymorphism in the promoter of the human gene for metallothionein IIA." Hum Genet 120(4): 553-560.

Most people generally ingest cadmium in their food. Cadmium that has accumulated in tissues induces the synthesis of metallothioneins (MTs) which are metal-binding proteins that bind tightly to cadmium to inhibit its renal toxicity. Individuals whose ability to induce the synthesis of MTs is low seem likely to be particularly susceptible to the toxic effects of cadmium. In this study, we analyzed the polymorphism of the promoter region of the gene for MT-IIA, the major species of MT in humans, in 119 adult Japanese subjects. We found that about 18% of the subjects had an A --> G single-nucleotide polymorphism in the core region of the promoter near the TATA box. A reporter-gene assay using HEK293 cells showed that replacement of A by G at position -5 reduced the efficiency of the cadmium-induced transcription of the gene for MT-IIA. This single-nucleotide polymorphism inhibited the binding of nuclear proteins to the core promoter region of the gene for MT-IIA. When the promoter region upstream of the TATA box was replaced by a sequence that contained three dioxin-responsive elements, the reporter-gene assay demonstrated that the A --> G single-nucleotide polymorphism resulted in a marked reduction in the rate of dioxin-induced transcription. These results suggest that the A --> G single-nucleotide polymorphism reduces the efficiency of those aspects of the transcription of the gene for MT-IIA that are controlled by general transcription factors.

Knapen, D., et al. (2005). "New metallothionein

mRNAs in Gobio gobio reveal at least three gene duplication events in cyprinid metallothionein evolution." Comp Biochem Physiol C Toxicol Pharmacol 140(3-4): 347-355.

This paper reports the identification and analysis of the primary structure of three novel metallothionein cDNA sequences in the gudgeon, Gobio gobio (Cyprinidae). Two different 180 bp coding regions were identified, resulting in two MT isoforms differing in one amino acid. The primary structure of the amino acid sequence was compared to other cyprinid MT sequences. Furthermore, two differently sized cDNAs were discovered in one of the two transcripts. We present a phylogenetic comparison of our sequences to other, previously published cyprinid MT gene sequences. Our analysis reveals an unexpected complexity in cyprinid MT evolution, with at least three gene duplication events. Differences and homologies between the evolution of cyprinid MT genes are compared to other teleost families. Finally, possible implications for metallothionein classification are discussed.

Knapen, D., et al. (2007). "Metallothionein gene and protein expression as a biomarker for metal pollution in natural gudgeon populations." Aquat Toxicol 82(3): 163-172.

Gudgeons (Gobio gobio) from historically Cd and Zn contaminated sites in Flanders (Belgium) were found to be resistant to elevated Cd levels. In previous work, this increased resistance was largely explained by increased metallothionein (MT) expression. Recently, environmental cleanup efforts resulted in a significant decrease in Cd concentrations in the surface water. In this study, we evaluated the use of hepatic metal and metallothionein (MT) concentrations as biomarkers of metal exposure before and after the cleanup. Hepatic MT mRNA levels were determined after the environmental metal levels decreased in order to assess the applicability of MT gene expression as an environmental biomarker in natural fish populations. Our data show that both metallothionein protein and gene expression have the potential to be sensitive biomarkers for metal exposure. Significant correlations were found (a) among accumulated metal concentrations and both MT protein and mRNA levels, and (b) between MT protein and mRNA levels. However, our data illustrated that while MT protein and gene expression give a quantitative picture of metal load at a single time point, quantitative information in natural populations cannot always be obtained when different time points (including different years) are compared, since MT gene and protein expression are affected by many other factors in addition to the metal load. Furthermore, the result of the environmental cleanup was reflected in a decrease of hepatic Cd concentrations. Zn remained the most important factor determining MT concentrations. Finally, two differently sized MT mRNAs were amplified to test the hypothesis that 3'-UTR length can offer a protective advantage in conditions of environmental stress. Our data provided no evidence to support this hypothesis. In contrast, the ratio of the long mRNA variant relative to total MT mRNA was surprisingly constant, and independent of exposure history.

Koizumi, S. and F. Otsuka (1994). "Nuclear proteins binding to the human metallothionein-IIA gene upstream sequences." Ind Health 32(4): 193-205.

Metallothionein genes are known to be transcriptionally regulated by a variety of factors such as heavy metals, glucocorticoids and cytokines, and have multiple regulatory elements in their 5'-flanking region. To study the interactions between these sequences and regulatory factors, HeLa cell nuclear proteins were analyzed by band-shift assay using a 95-base pair (bp) DNA probe containing a part of the human MT-IIA gene upstream sequences. Consequently, two Zn-dependent DNA-binding proteins were detected. One of these showed properties almost identical with those of zinc regulatory factor (ZRF), which had been detected using an oligonucleotide probe containing the metal responsive element (MRE); namely, this protein is activated only by Zn, and requires not only MRE but also its flanking sequences for optimal DNA-binding. The other protein appears to be Sp1, based on its recognition sequences specificity. In addition, by Southwestern blotting analysis of nuclear extracts using the 95-bp probe or MRE oligonucleotide probe, we detected a Zn-dependent DNA-binding protein with a molecular mass of 116 kDa, which is likely to be ZRF. Analysis of HeLa cell nuclear proteins fractionated by glycerol gradient centrifugation showed that ZRF is distinct from another MRE-binding protein, MREBP.

Koizumi, S., et al. (1991). "A nuclear factor that interacts with metal responsive elements of a human metallothionein gene." Chem Biol Interact 80(2): 145-157.

Metallothioneins (MTs) are low molecular weight heavy metal-binding proteins which are known to play a major role in heavy metal detoxification and understanding of their regulatory mechanism is toxicologically important. Expression of MT genes is induced by heavy metals and metal responsive elements (MREs) upstream of MT genes are essential for the transcriptional activation. By several types of mobility shift assay with 32P-labeled oligonucleotide probes, we detected HeLa cell nuclear as well as cytoplasmic factors that bind to MRE sequences of human MTIIA (hMTIIA) gene. One of the nuclear factors, which gives stronger signal than others, was further characterized. Competition experiments showed that the nuclear factor (named MREBP) specifically

recognizes MREs of hMTIIA gene. EDTA abolished the binding of MREBP to MRE, suggesting that a divalent cation(s) is required for the complex formation. Also in blotting experiments with HeLa nuclear extract and the [32P]MRE probes an EDTA-sensitive 95k protein band, which possibly represents MREBP, was detected.

Koizumi, S., et al. (1999). "Transcriptional activity and regulatory protein binding of metal-responsive elements of the human metallothionein-IIA gene." Eur J Biochem 259(3): 635-642.

Multiple copies of a cis-acting DNA element, metal-responsive element (MRE) are required for heavy metal-induced transcriptional activation of mammalian metallothionein genes. To approach the regulatory mechanism mediated by these multiple elements, we studied the properties of seven MREs located upstream of the human metallothionein-IIA (hMT-IIA) gene in detail. Transfection assays of reporter gene constructs each containing one of these MREs as the promoter element revealed that only four MREs can mediate zinc response. With respect to the distribution of active MREs over the promoter region, the hMT-IIA gene is largely different from the mouse metallothionein-I gene, suggesting that MRE arrangement is not an important factor for metal regulation. Experiments using various model promoters showed that multiple MRE copies act highly synergistically, supporting the biological significance of the multiplicity. Only the four active MREs efficiently bound the purified transcription factor human MTF-1, and MRE mutants defective in binding this protein lost the ability to support zinc-induced reporter gene expression, strongly suggesting that the direct interaction between human MTF-1 and a set of the selected MREs plays the major role in heavy metal regulation. In protein/DNA binding reactions in vitro, the purified human MTF-1 was activated by zinc but not by other metallothionein-inducing heavy metals, supporting the idea that zinc is the direct modulator of human MTF-1.

Koizumi, S., et al. (1992). "A nuclear factor that recognizes the metal-responsive elements of human metallothionein IIA gene." J Biol Chem 267(26): 18659-18664.

Expression of metallothionein (MT) genes is regulated by heavy metals mainly at the transcriptional level, via cis-acting elements called the metal-responsive elements (MREs). A HeLa cell nuclear factor that recognizes MREs of the human MTIIA (hMTIIA) gene, MREBP, was characterized. Mobility shift assay and DNase I footprinting experiments showed that MREBP binds specifically to several MREs present upstream of the hMTIIA gene. Cadmium and zinc ions inhibited binding of MREBP to a MRE at high concentrations, suggesting a role of MREBP in the negative regulation of the hMTIIA gene. MREBP was partially purified by passing the HeLa nuclear extract over heparin-agarose, Sephacryl S-300, and MRE-Sepharose affinity columns. Blotting experiments showed that a polypeptide with an M(r) of 112,000 is responsible for the MREBP activity.

Koizumi, S., et al. (1992). "Zinc-specific activation of a HeLa cell nuclear protein which interacts with a metal responsive element of the human metallothionein-IIA gene." Eur J Biochem 210(2): 555-560.

Transcription of metallothionein genes is activated by heavy metals such as zinc and cadmium, and a DNA element called metal responsive element (MRE) is essential for this process. By mobility-shift assay, we identified a HeLa-cell nuclear protein which specifically binds to MREa of human metallothionein-IIA gene. This protein, named ZRF (zinc-regulatory factor), is present in the cells untreated with heavy metals. Zinc is essential for, and increases in a dose-dependent manner, the binding of ZRF to MREa. Other heavy metals which can also induce metallothioneins, including cadmium, copper and mercury, do not activate ZRF. A MREa-containing oligonucleotide that can bind ZRF confers heavy metal-inducibility to a heterologous promoter, suggesting that ZRF is a zinc-dependent transcriptional activator. In addition to the MRE core sequence, the surrounding sequences are also important for both ZRF binding in vitro, and zinc-dependent transcriptional activation in vivo. MREa by itself responds not only to zinc but also to other metallothionein-inducing heavy metals, indicating that the ZRF protein, not the MREa sequence, is responsible for the zinc specificity.

Kojima, S., et al. (1998). "Molecular cloning and expression of the canine metallothionein-III gene." Can J Vet Res 62(2): 148-151.

We have isolated and determined the complete nucleotide sequence of canine metallothionein-III (MT-III) cDNA. The predicted amino acid sequence of the canine MT-III showed a high homology (93%, 87% identity) to that of human and mouse MT-III. The canine MT-III had 2 insertions relative to known mammalian MT-I and MT-II: a threonine after the 4th amino acid and a block of 6 amino acids near the carboxyl terminus. Expression of the canine MT-III mRNA was found exclusively in the central nervous system, where neurons in the olfactory bulb, hippocampus and cerebral cortex showed predominant signals.

Koropatnick, J., et al. (1983). "Mouse hepatic metallothionein-I gene cleavage by micrococcal nuclease is enhanced after induction by cadmium." Nucleic Acids Res 11(10): 3255-3267.

Micrococcal nuclease has been shown to preferentially cleave chromatin in the region of genes actively engaged in transcription. We have used this

preferential cleavage to show that the metallothionein (MT) gene in adult mouse liver, when induced to produce mRNA by injection of cadmium, becomes more susceptible to nuclease cleavage. However, the MT gene in uninduced liver, and the alphafoetal protein (AFP) gene in both induced and uninduced liver, remain relatively resistant to nuclease cleavage. The AFP gene is not normally expressed in cadmium induced or uninduced liver. Thus, susceptibility of genes to nuclease cleavage appears to rise with increasing transcription of the gene.

Koropatnick, J. and J. D. Duerksen (1987). "Nuclease sensitivity of alpha-fetoprotein, metallothionein-1, and immunoglobulin gene sequences in mouse during development." Dev Biol 122(1): 1-10.

The production of alpha-fetoprotein (AFP) and metallothionein-1 (MT-1) in mouse tissues follows a well-defined developmental pattern. The genes for these proteins are highly transcribed in embryo liver but transcribed at a very low rate in adult liver and in brain at all stages of development. A dot hybridization procedure was defined for quantitative screening for AFP, MT-1, immunoglobulin, and satellite DNA sequences to determine the relative degree of micrococcal nuclease sensitivity of these DNA sequences in fetal, newborn, and adult liver and brain, and the visceral yolk sac of the embryo. It was found that, for the DNA sequences assayed, three distinct chromatin conformations exist. DNA that does not code for protein (satellite DNA) was highly resistant to nuclease cleavage. DNA that codes for protein, but is not available for transcription (unrearranged immunoglobulin (C mu) genes in brain, liver, and yolk sac) was fourfold more sensitive to cleavage than were satellite DNA sequences. A further sevenfold increase in nuclease sensitivity was detected in genes actively being transcribed (MT-1 and AFP genes in embryo liver). Quiescent MT-1 and AFP genes were intermediate in nuclease-sensitivity between active genes and unrearranged C mu genes. These data indicate that MT-1 and AFP genes are permanently established in a nuclease-sensitive chromatin conformation early in liver development, and that conformation is maintained regardless of the degree of transcription of the genes. A second, reversible change in chromatin structure occurs in step with changes in the degree of developmentally regulated expression of AFP and MT-1 genes.

Koropatnick, J., et al. (1985). "Acute treatment of mice with cadmium salts results in amplification of the metallothionein-1 gene in liver." Nucleic Acids Res 13(15): 5423-5439.

A variety of genes have been shown to change copy number during development, including rRNA genes in amphibians and chorion proteins in insects. Dihydrofolate reductase and metallothionein-1 (MT-1) genes are present in high copy number in cultured mammalian cells subjected to low levels of agents that will select for cells with amplified copies of specific genes. Recent studies have shown that the metallothionein-1 gene in mouse liver is regulated at the transcriptional level by treatment with heavy metals. We report here that, at cadmium concentrations 5 to 10-fold higher than that required to induce maximal transcription of the MT-1 gene, there is a 2 to 3-fold increase in MT-1 gene concentration in liver nuclear DNA by 6 hours after induction, and extra copies persist up to 3 weeks in the absence of further heavy metal treatment. The extra MT-1 gene copies that appear 6 hours after cadmium treatment are in a conformation that renders them relatively nuclease insensitive.

Krzeslak, A., et al. (2013). "Effect of metallothionein 2A gene polymorphism on allele-specific gene expression and metal content in prostate cancer." Toxicol Appl Pharmacol 268(3): 278-285.

Metallothioneins (MTs) are highly conserved, small molecular weight, cysteine rich proteins. The major physiological functions of metallothioneins include homeostasis of essential metals Zn and Cu and protection against cytotoxicity of heavy metals. The aim of this study was to determine whether there is an association between the -5 A/G single nucleotide polymorphism (SNP; rs28366003) in core promoter region and expression of metallothionein 2A (MT2A) gene and metal concentration in prostate cancer tissues. MT2A polymorphism was determined by the polymerase chain reaction-restriction fragment length polymorphism technique (PCR-RFLP) using 412 prostate cancer tissue samples. MT2A gene expression analysis was performed by real-time RT-PCR method. A significant association between rs28366003 genotype and MT2A expression level was found. The average mRNA level was found to be lower among minor allele carriers (the risk allele) than average expression among homozygotes for the major allele. Metal levels were analyzed by flamed atomic absorption spectrometer system. Highly statistically significant associations were detected between the SNP and Cd, Zn, Cu and Pb levels. The results of Spearman's rank correlation showed that the expressions of MT2A and Cu, Pb and Ni concentrations were negatively correlated. On the basis of the results obtained in this study, we suggest that SNP polymorphism may affect the MT2A gene expression in prostate and this is associated with some metal accumulation.

Kubo, T., et al. (2016). "Upregulations of metallothionein gene expressions and tolerance to heavy metal toxicity by three dimensional cultivation of HepG2 cells on VECELL 3-D inserts." J Toxicol Sci 41(1): 147-153.

The VECELL 3-D insert is a new culture

scaffold consisting of collagen-coated ePTFE (expanded polytetrafluoroethylene) mesh. We analyzed the effects of VECELL 3-D inserts on the functionality of HepG2, a human hepatocellular carcinoma cell line. HepG2 cells cultured on VECELL 3-D inserts maintained a round shape, while those cultured on a standard culture plate or collagen-coated cell culture plate showed a flattened and cubic epithelial-like shape. HepG2 cells cultured on VECELL 3-D inserts had showed upregulated expression of metallothionein genes and in turn a higher tolerance to toxicity induced by heavy metals. These results suggest that HepG2 cell functions were changed by the cell morphology that is induced by culturing on a VECELL 3-D insert.

Kumar, K. S., et al. (2005). "Copper alone, but not oxidative stress, induces copper-metallothionein gene in Neurospora crassa." FEMS Microbiol Lett 242(1): 45-50.

Two metal response elements, flanking an antioxidant response element, were identified in regions upstream (-3730 bp) to copper metallothionein (CuMT) gene of Neurospora crassa. Presence of copper in culture media, but not of pro-oxidants like H2O2 or menadione, induced CuMT gene expression that could not be completely abolished by antioxidants such as N-acetyl cysteine and ascorbic acid. Gel shift assays revealed the ability of nuclear extracts from copper induced cultures to bind PCR-amplified metal response or antioxidant response elements. Similar observations could not be made with cultures exposed either to pro-oxidants or antioxidants. These results differentiate between CuMT gene induction by copper from antioxidant functions associated with the identified upstream elements.

Kumar, K. S., et al. (2006). "Characterization of calcineurin-dependent response element binding protein and its involvement in copper-metallothionein gene expression in Neurospora." Biochem Biophys Res Commun 345(3): 1010-1013.

In continuation of our recent observations indicating the presence of a lone calcineurin-dependent response element (CDRE) in the -3730bp upstream region of copper-induced metallothionein (CuMT) gene of Neurospora [K.S. Kumar, S. Dayananda, C. Subramanyam, Copper alone, but not oxidative stress, induces copper-metallothionein gene in Neurospora crassa, FEMS Microbiol. Lett. 242 (2005) 45-50], we isolated and characterized the CDRE-binding protein. The cloned upstream region of CuMT gene was used as the template to specifically amplify CDRE element, which was immobilized on CNBr-activated Sepharose 4B for use as the affinity matrix to purify the CDRE binding protein from nuclear extracts obtained from Neurospora cultures grown in presence of copper. Two-dimensional gel electrophoresis of the affinity purified protein revealed the presence of a single 17kDa protein, which was identified and characterized by MALDI-TOF. Peptide mass finger printing of tryptic digests and analysis of the 17kDa protein matched with the regulatory beta-subunit of calcineurin (Ca(2+)-calmodulin dependent protein phosphatase). Parallel identification of nuclear localization signals in this protein by in silico analysis suggests a putative role for calcineurin in the regulation of CuMT gene expression.

Kumari, S. and S. Das (2019). "Expression of metallothionein encoding gene bmtA in biofilm-forming marine bacterium Pseudomonas aeruginosa N6P6 and understanding its involvement in Pb(II) resistance and bioremediation." Environ Sci Pollut Res Int 26(28): 28763-28774.

The genetic basis and biochemical aspects of heavy metal endurance abilities have been precisely studied in planktonic bacteria; however, in nature, bacteria mostly grows as surface-attached communities called biofilms. A hallmark trait of biofilm is increased resistance to heavy metals compared with the resistance of planktonic bacteria. A proposed mechanism that contributes to this increased resistance is the enhanced expression of metal-resistant genes. bmtA gene coding for metallothionein protein is one such metal-resistant gene found in many bacterial spp. In the present study, lead (Pb) remediation potential of a biofilm-forming marine bacterium Pseudomonas aeruginosa N6P6 was explored. Biofilm-forming marine bacterium P. aeruginosa N6P6 possess bmtA gene and shows resistance towards many heavy metals, i.e., Pb, Cd, Hg, Cr, and Zn. The expression of metallothionein encoding gene bmtA is significantly high in 48-h-old biofilm culture (11. 4 fold) followed by 24-h-old biofilm culture of P. aeruginosa N6P6 (4.7 fold) ($P < 0.05$). However, in the case of planktonically grown culture of P. aeruginosa N6P6, the highest expression of bmtA gene was observed in 24-h-old culture. The expression of bmtA also increased significantly with increase in Pb concentration up to 800 ppm. CSLM analysis indicated significant reduction in the raw integrated density of biofilm-associated lipids and polysaccharides (PS) of P. aeruginosa N6P6 biofilm grown in Pb (sub-lethal concentration)-amended medium ($P < 0.05$), whereas no significant reduction was observed in the raw integrated density of EPS-associated protein. The role of bmtA gene as Pb(II)-resistant determinant was characterized by overexpressing the bmtA gene derived from P. aeruginosa N6P6 in Escherichia coli BL21(DE3). ESI-MS and SDS-PAGE analyses validated the presence of 11.5-kDa MT protein isolated from Pb(II)-induced recombinant E. coli BL21(DE3) harboring bmtA gene.

Kupper, U., et al. (1990). "Expression of tyrosinase in vegetative cultures of Neurospora crassa transformed with a metallothionein promoter/protyrosinase fusion

gene." Curr Genet 18(4): 331-335.

Wild-type Neurospora crassa, strain Singapore, was transformed with a N. crassa metallothionein promoter/protyrosinase fusion gene. Transformants produced tyrosinase during vegetative growth, as determined by Western analyses and activity assays. This is in sharp contrast to wild-type strains, where this enzyme is only expressed in situations of starvation or sexual differentiation. Complete integration of a 400 bp metallothionein promoter-fragment leads to constitutive expression of protyrosinase, whereas a 3.6 kb promoter-fragment conferred copper inducibility on the reporter gene in four transformants. A transformant with high constitutive tyrosinase levels was able to produce melanin on complete medium agar plates supplemented with 1 mg/ml L-tyrosine.

Kurita, H., et al. (2013). "Prenatal zinc deficiency-dependent epigenetic alterations of mouse metallothionein-2 gene." J Nutr Biochem 24(1): 256-266.

Zinc (Zn) deficiency in utero has been shown to cause a variety of disease states in children in developing countries, which prompted us to formulate the hypothesis that fetal epigenetic alterations are induced by zinc deficiency in utero. Focusing on metallothionein (MT), a protein that contributes to Zn transport and homeostasis, we studied whether and how the prenatal Zn status affects gene expression. Pregnant mice were fed low-Zn (IU-LZ, 5.0 mug Zn/g) or control (IU-CZ, 35 mug Zn/g) diet ad libitum from gestation day 8 until delivery, with a regular diet thereafter. Bisulfite genomic sequencing for DNA methylation and chromatin immunoprecipitation assay for histone modifications were performed on the MT2 promoter region. We found that 5-week-old IU-LZ mice administered cadmium (Cd) (5.0 mg/kg b.w.) have an elevated abundance of MT2 mRNA compared with IU-CZ mice. Alteration of histone modifications in the MT2 promoter region having metal responsive elements (MREs) was observed in 1-day-old and 5-week-old IU-LZ mice compared with IU-CZ mice. In addition, prolongation of MTF1 binding to the MT2 promoter region in 5-week-old IU-LZ mice upon Cd exposure is considered to contribute to the enhanced MT2 induction. In conclusion, we found for the first time that Zn deficiency in utero induces fetal epigenetic alterations and that these changes are being stored as an epigenetic memory until adulthood.

Labbe, S., et al. (1991). "A nuclear factor binds to the metal regulatory elements of the mouse gene encoding metallothionein-I." Nucleic Acids Res 19(15): 4225-4231.

The ability of vertebrate metallothionein (MT) genes to be induced by heavy metals is controlled by metal regulatory elements (MREs) present in the promoter in multiple, non-identical copies. The binding specificity of the mouse L-cell nuclear factor(s) that interact with the element MREd of the mouse MT-I gene was analyzed by in vitro footprinting, protein blotting, and UV cross-linking assays. In vitro footprinting analyses revealed that synthetic oligodeoxynucleotides (oligomers) corresponding to the metal regulatory elements MREa, MREb, MREc, MREd and MREe of the mouse MT-I gene, as well as the MRE4 of the human MT-IIA gene and the MREa of the trout MT-B gene, all competed for the nuclear protein species binding to the MREd region of the mouse MT-I gene, the MREe oligomer being the weakest competitor. In addition, protein blotting experiments revealed that a nuclear protein of 108 kDa, termed metal element protein-1 (MEP-1), which specifically binds with high affinity to mouse MREd, binds with different affinities to the other mouse MRE elements, mimicking their relative transcriptional strength in vivo: MREd greater than or equal to MREa = MREc greater than MREb greater than MREe greater than MREf. Similarly, human MRE4 and trout MREa bind to MEP-1. A protein similar in size to MEP-1 was also detected in HeLa-cell nuclear extracts. In UV cross-linking experiments the major protein species, complexed with mouse MREd oligomers, migrated on a denaturating gel with an apparent Mr of 115,000 and was detected using each of the mouse MRE oligomers tested. These results show that a mouse nuclear factor can bind to multiple MREs in mouse, trout, and human MT genes.

Lachke, S. A., et al. (2000). "Phenotypic switching in Candida glabrata involves phase-specific regulation of the metallothionein gene MT-II and the newly discovered hemolysin gene HLP." Infect Immun 68(2): 884-895.

Although Candida glabrata has emerged in recent years as a major fungal pathogen, there have been no reports demonstrating that it undergoes either the bud-hypha transition or high-frequency phenotypic switching, two developmental programs believed to contribute to the pathogenic success of other Candida species. Here it is demonstrated that C. glabrata undergoes reversible, high-frequency phenotypic switching between a white (Wh), light brown (LB), and dark brown (DB) colony phenotype discriminated on an indicator agar containing 1 mM CuSO(4). Switching regulates the transcript level of the MT-II metallothionein gene(s) and a newly discovered gene for a hemolysin-like protein, HLP. The relative MT-II transcript levels in Wh, LB, and DB cells grown in the presence of CuSO(4) are 1:27:81, and the relative transcript levels of HLP are 1:20:35. The relative MT-II and HLP transcript levels in cells grown in the absence of CuSO(4) are 1:20:30 and 1:20:25, respectively. In contrast, switching has little or no effect on the transcript levels of the genes MT-I, AMT-I, TRPI, HIS3,

EPAI, and PDHI. Switching of C. glabrata is not associated with microevolutionary changes identified by the DNA fingerprinting probe Cg6 and does not involve tandem amplification of the MT-IIa gene, which has been shown to occur in response to elevated levels of copper. Finally, switching between Wh, LB, and DB occurred in all four clinical isolates examined in this study. As in Candida albicans, switching in C. glabrata may provide colonizing populations with phenotypic plasticity for rapid responses to the changing physiology of the host, antibiotic treatment, and the immune response, through the differential regulation of genes involved in pathogenesis. More importantly, because C. glabrata is haploid, a mutational analysis of switching is now feasible.

Ladhar-Chaabouni, R., et al. (2009). "Cloning and characterization of cDNA probes for the analysis of metallothionein gene expression in the Mediterranean bivalves: Ruditapes decussatus and Cerastoderma glaucum." Mol Biol Rep 36(5): 1007-1014.

cDNA probes have been developed for subsequent use in monitoring the cadmium exposure of the clam Ruditapes decussatus and the cockle Cerastoderma glaucum using metallothionein (MT) gene expression in different tissues of these species. Two partial MT cDNAs were isolated from Ruditapes decussatus and Cerastoderma glaucum. The identification of the nucleotide sequences showed that the cDNAs consist of 480 bp coding 72 amino acid proteins containing 21 cysteine residues organized in Cys-X-Cys motifs as classically described for MTs. The induction of MT gene expression in CdCl(2) treated bivalves was confirmed by dot blot analysis and suggests a potential specific tissue expression rate.

Lai, Y., et al. (2010). "Silencing the Metallothionein-2A gene induces entosis in adherent MCF-7 breast cancer cells." Anat Rec (Hoboken) 293(10): 1685-1691.

The presence of a live cell cohabiting within another cell has fascinated scientists for many decades. Far from being a spurious event, many have attempted to uncover the molecular mechanism underlying this phenomenon. In this study, we observed anchorage-dependent MCF-7 cells internalizing neighboring epithelial cells (entosis) after siRNA-mediated silencing of the Metallothionein-2A (MT-2A) gene. MTs belong to a family of low-molecular weight proteins, which bind metal ions endogenously and its over-expression has been reported in a variety of cancers that include breast, prostate, and colon. We provide microscopic evidence at light and ultrastructural levels of the occurrence of entosis after altering MT expression in a subpopulation of MCF-7 breast cancer cells by silencing the MT-2A gene. Our results demonstrate that adheren junctions may play important roles in the formation of cell-in-cell cytostructure after MT-2A gene downregulation and the entotic process does not appear to involve genes associated with autophagy. Interiorized cells often underwent lysosomal degradation within the cytoplasmic body of the engulfing cell. It would appear that a subset of breast cancer cells could die via entosis after MT-2A gene silencing.

Lambros, M. P., et al. (2002). "Targeting hepatocytes with liposomal interferon-alpha: effect on metallothionein gene induction." Res Commun Mol Pathol Pharmacol 112(1-4): 50-58.

Interferon-alpha (INF-alpha) is the only effective drug for the treatment of chronic hepatitis B. However it can produce severe side effects during treatment. Encapsulation of INF-alpha in liposomes may reduce the side effects and enhance its therapeutic activity. We evaluated the activity of free (nonencapsulated) and liposome-encapsulated INF-alpha on in vitro cultured Chang liver cells by measuring the metallothionein gene (MT-IIA). INF-alpha was encapsulated in different liposomal formulations, Dimyristoylphosphatidylcholine (DMPC), Dioleyl-phosphatidyl-ethanolamine/Dimyristoylphosphatidylcholine (DOPE/DMPC) and DOPE/Dimyristoylphosphatidylglycerol (DOPE/DMPG). Chang liver cells were incubated for 10 hours with 100 units/ml of free or one of the aforementioned liposomal INF-alpha formulations. We also evaluated the extended-time effects of DMPC liposomal formulations of INF-alpha and the non-encapsulated (free) INF-alpha on Chang liver cells after 12, 24 and 36 h of incubation. Total RNA was extracted and signals on Northern blots were densitometrically compared following hybridization with MT-IIA and beta actin probes. All INF-alpha formulations (free and liposomal) induced higher MT-IIA gene levels compared to non-treated control cells. Levels of MT-IIA mRNA expression were 80.9, 73.6, 43.9, and 35.3% over the control for the free, DOPE/DMPG, DMPC and DOPE/DMPC liposomal INF-alpha, respectively. The ratios of MT-IIA mRNA amounts expressed after the Chang liver cells were incubated with INF-alpha encapsulated in DMPC liposomes and the MT-IIA mRNA expressed after incubation with nonencapsulated INF-alpha are 0.7, 0.52 and 0.82 at 12, 24, and 36 hours, respectively. The results indicate that the MT-IIA mRNA level depends on the liposomal formulation of INF-alpha, and the sustained-time effect of the INF-alpha encapsulated in DMPC liposomes is parallel to that of nonencapsulated INF-alpha over a period of 36 hours.

Lanfranco, L., et al. (2002). "Differential expression of a metallothionein gene during the presymbiotic versus the symbiotic phase of an arbuscular mycorrhizal fungus." Plant Physiol 130(1): 58-67.

A full-length cDNA encoding a

metallothionein (MT)-like polypeptide, designated GmarMT1, was identified in an expressed sequence tag collection from germinated spores of the arbuscular mycorrhizal fungus Gigaspora margarita (BEG34). The GmarMT1 gene is composed of two exons separated by an 81-bp intron. It codes for a 65-amino acid polypeptide comprising a plant type 1 MT-like N-terminal domain and a C-terminal domain that is most closely related to an as-yet-uncharacterized fungal MT. As revealed by heterologous complementation assays in yeast, GmarMT1 encodes a functional polypeptide capable of conferring increased tolerance against Cd and Cu. The GmarMT1 RNA is expressed in both presymbiotic spores and symbiotic mycelia, even in the absence of metal exposure, but is significantly less abundant in the latter stage. An opposite pattern was observed upon Cu exposure, which up-regulated GmarMT1 expression in symbiotic mycelia but not in germinated spores. Together, these data provide the first evidence, to our knowledge, for the occurrence in an arbuscular mycorrhizal fungus of a structurally novel MT that is modulated in a metal and life cycle stage-dependent manner and may afford protection against heavy metals (and other types of stress) to both partners of the endomycorrhizal symbiosis.

Laplaze, L., et al. (2002). "Symbiotic and non-symbiotic expression of cgMT1, a metallothionein-like gene from the actinorhizal tree Casuarina glauca." Plant Mol Biol 49(1): 81-92.

A clone for a type 1 metallothionein (cgMT1) was isolated from a Casuarina glauca nodule cDNA library. The corresponding gene belongs to a small family and is highly expressed in roots and nitrogen-fixing nodules, whereas low expression was observed in aerial parts of the plant. The promoter region of cgMT1 was isolated and fused to the beta-glucuronidase (gus) gene. Transgenic Casuarinaceae plants showed that the cgMT1 promoter was most active in roots and in the oldest region of the shoot. In situ hybridization indicated that in nodules cgMT1 transcript is present in mature Frankia-infected cells and in the pericycle. Possible roles for cgMT1 in symbiotic and nonsymbiotic tissues are discussed.

LaRochelle, O., et al. (2008). "Nuclear factor-1 and metal transcription factor-1 synergistically activate the mouse metallothionein-1 gene in response to metal ions." J Biol Chem 283(13): 8190-8201.

Metal activation of metallothionein (MT) gene transcription is dependent on the presence of metal regulatory elements (MREs), which are present in five non-identical copies (MREa through MREe) in the promoter of the mouse MT-1 gene and on the capacity of metal transcription factor-1 (MTF-1) to bind to the MREs in the presence of zinc. We detected a protein, distinct from MTF-1, specifically binding to the MREc region. DNA binding competition experiments using synthetic oligonucleotides and specific anti-NF1 antibodies showed that this protein binds to an NF1 site overlapping the MREc element as well as to a second site upstream of the Sp1a site and corresponds to NF1 or a related protein. Transfection experiments showed that loss of the two NF1 sites decreased metal-induced MT promoter activity by 55-70% in transiently transfected cells and almost completely abrogated metal and tert-butylhydroquinone (tBHQ) induction in stably transfected cells. Similarly, expression of an inactive NF1 protein strongly inhibited MT-1 promoter activity. Using synthetic promoters containing NF1 and MRE sites fused to a minimal MT promoter, we showed that these NF1 sites did not confer metal induction but enhanced metal-induced promoter activity. Chromatin immunoprecipitation assays confirmed that NF1 binds to the mouse MT-1 promoter in vivo and showed that NF1 binding is zinc-inducible. In addition, zinc-induced NF1 DNA binding was MTF-1-dependent. Taken together, these studies show that NF1 acts synergistically with MTF-1 to activate the mouse MT-1 promoter in response to metal ions and tert-butylhydroquinone and contributes to maximal activation of the gene.

Laukens, D., et al. (2009). "Human metallothionein expression under normal and pathological conditions: mechanisms of gene regulation based on in silico promoter analysis." Crit Rev Eukaryot Gene Expr 19(4): 301-317.

Metallothioneins (MTs) are ubiquitous metal-binding proteins that have been highly conserved throughout evolution. Although their physiological function is not completely understood, they are involved in diverse processes including metal homeostasis and detoxification, the oxidative stress response, inflammation, and cell proliferation. Te human MT gene family consists of at least 18 isoforms, containing pseudogenes as well as genes encoding functional proteins. Most of the MT isoforms can be induced by a wide variety of substances, such as metals, cytokines, and hormones. Different cell types express discrete MT isoforms, which reflects the specifically adapted functions of MTs and a divergence in their regulation. Te aberrant expression of MTs has been described in a number of diseases, including Crohn's disease, cancer, Alzheimer's disease, amyotrophic lateral sclerosis, Menkes disease, and Wilson's disease. Therefore, a thorough understanding of MT gene regulation is imperative. To date, the transcriptional regulation of MTs has primarily been studied in mice. While only four murine MT isoforms exist, the homology between murine and human MTs allows for the evaluation of the regulatory regions in their respective promoters. Here, we review the aberrant expression of MTs in human diseases and the mechanisms that regulate MT1 expression based on an

in silico evaluation of transcription factor binding sites.
Le Beau, M. M., et al. (1985). "Metallothionein gene cluster is split by chromosome 16 rearrangements in myelomonocytic leukaemia." Nature 313(6004): 709-711.

The metallothioneins (MTs) are a family of proteins of low relative molecular mass which bind heavy-metal ions. MTs exist in several molecular forms (MT-I, MT-II) and are encoded by a multi-gene family containing at least 14 closely related genes and pseudogenes. These proteins function in the regulation of trace-metal metabolism, the storage of these ions in the liver, and as a protective mechanism against heavy-metal toxicity. Somatic cell hybridization has shown that most MT genes, including the functional MT genes (MT1A, MT1B, MT2A), lie on human chromosome 16. Using in situ hybridization, we have now localized the MT genes to band q22 of chromosome 16. This chromosomal band is also a breakpoint in two specific rearrangements, the inv(16)(p13q22) and t(16; 16)(p13;q22) rearrangements, found in a subgroup of patients with acute myelomonocytic leukaemia (AMML). Hybridization of a MT probe to malignant cells from two patients with an inv(16) showed labelled sites on both arms of the inverted chromosome, indicating that the breakpoint at 16q22 splits the MT gene cluster. Similar results were obtained when this probe was hybridized to metaphase cells from two patients with a t(16; 16). These results suggest that the MT genes or their regulatory regions may function as an 'activating' sequence for an as yet unidentified cellular gene located at 16p13.
Leadon, S. A. and M. M. Snowden (1988). "Differential repair of DNA damage in the human metallothionein gene family." Mol Cell Biol 8(12): 5331-5338.

We studied the repair of UV- and aflatoxin B1 (AFB1)-induced damage in the human metallothionein (hMT) gene family. After exposure to either UV or AFB1, DNA damage was initially repaired faster in the DNA fragments containing the transcribed hMT-IA, hMT-IE, and hMT-IIA genes than in the genome overall. By 6 h posttreatment, there was at least twice as much repair in these genes as in the rest of the genome. Repair of UV damage in the hMT-IB gene, which shows cell-type specific expression, and in the hMT-IIB gene, which is a nontranscribed processed pseudogene, was about the same as in the rest of the genome, whereas repair of AFB1-induced damage was deficient in these two genes. Inducing transcription of the three expressed hMT genes with CdCl2 or of only the hMT-IIA gene with dexamethasone increased the initial rate of repair in the induced genes another twofold over the rate observed when they were transcribed at a basal level. The rates of repair in the hMT-IB and hMT-IIB genes were not altered by these inducing treatments. Transcription of the hMT genes was transiently inhibited after UV irradiation. Inducing transcription of the genes did not shorten this UV-induced delay. Thus, the efficiency of repair of damage in a DNA sequence is dependent on the level of transcriptional activity associated with that sequence. However, an increased efficiency in repair of a gene itself is not necessarily coupled to recovery of its transcription after DNA damage.
Lee, D. K., et al. (1999). "Identification of a signal transducer and activator of transcription (STAT) binding site in the mouse metallothionein-I promoter involved in interleukin-6-induced gene expression." Biochem J 337 (Pt 1): 59-65.

Mechanisms of regulation of mouse metallothionein (MT)-I gene expression in response to bacterial endotoxin-lipopolysaccharide (LPS) were examined. Northern blot analysis of hepatic MT-I mRNA in interleukin (IL)-6 or tumour necrosis factor (TNF)-receptor type I knock-out mice demonstrated that IL-6, not TNF-alpha, is of central importance in mediating hepatic MT-I gene expression in vivo after LPS injection. In vivo genomic footprinting of the MT-I promoter demonstrated a rapid increase, after LPS injection, in the protection of several guanine residues in the -250 to -300 bp region of the MT-I promoter. The protected bases were within sequences which resemble binding sites for the signal transducers and activators of transcription (STAT) transcription factor family. Electrophoretic mobility-shift assays using oligonucleotides from footprinted MT-I promoter regions showed that injection of LPS resulted in a rapid increase in the specific, high-affinity, in vitro binding of STAT1 and STAT3 to a binding site at -297 bp (TTCTCGTAA). Western blotting of hepatic nuclear proteins showed that the time-course for changes of total nuclear STAT1 and STAT3 after LPS injection paralleled the increased complex formation in vitro using this oligonucleotide, and binding was specifically competed for by a functional STAT-binding site from the rat alpha2-macroglobulin promoter. Furthermore, the MT-I promoter -297 bp STAT-binding site conferred IL-6 responsiveness in the context of a minimal promoter in transient transfection assays using HepG2 cells. This study suggests that the effects of LPS on hepatic MT-I gene expression are mediated by IL-6 and involve the activation of STAT-binding to the proximal promoter.
Lee, J. Y., et al. (2018). "Effect of Metallothionein-III on Mercury-Induced Chemokine Gene Expression." Toxics 6(3).

Mercury compounds are known to cause central nervous system disorders; however the detailed molecular mechanisms of their actions remain unclear. Methylmercury increases the expression of several chemokine genes, specifically in the brain, while

metallothionein-III (MT-III) has a protective role against various brain diseases. In this study, we investigated the involvement of MT-III in chemokine gene expression changes in response to methylmercury and mercury vapor in the cerebrum and cerebellum of wild-type mice and MT-III null mice. No difference in mercury concentration was observed between the wild-type mice and MT-III null mice in any brain tissue examined. The expression of Ccl3 in the cerebrum and of Cxcl10 in the cerebellum was increased by methylmercury in the MT-III null but not the wild-type mice. The expression of Ccl7 in the cerebellum was increased by mercury vapor in the MT-III null mice but not the wild-type mice. However, the expression of Ccl12 and Cxcl12 was increased in the cerebrum by methylmercury only in the wild-type mice and the expression of Ccl3 in the cerebellum was increased by mercury vapor only in the wild-type mice. These results indicate that MT-III does not affect mercury accumulation in the brain, but that it affects the expression of some chemokine genes in response to mercury compounds.

Lee, S., et al. (2015). "Cloning metallothionein gene in Zacco platypus and its potential as an exposure biomarker against cadmium." Environ Monit Assess 187(7): 447.

Zacco platypus, pale chub, is an indigenous freshwater fish of East Asia including Korea and has many useful characteristics as indicator species for water pollution. While utility of Z. platypus as an experimental species has been recognized, genetic-level information is very limited and warrants extensive research. Metallothionein (MT) is widely used and well-known biomarker for heavy metal exposure in many experimental species. In the present study, we cloned MT in Z. platypus and evaluated its utility as a biomarker for metal exposure. For this purpose, we sequenced complete complementary DNA (cDNA) of MT in Z. platypus and carried out phylogenetic analysis with its sequences. The transcription-level responses of MT gene following the exposure to CdCl2 were also assessed to validate the utility of this gene as an exposure biomarker. Analysis of cDNA sequence of MT gene demonstrated high conformity with those of other fish. MT messenger RNA (mRNA) expression and enzymatic MT content significantly increased following CdCl2 exposure in a concentration-dependent manner. The level of CdCl2 that resulted in significant MT changes in Z. platypus was within the range that was reported from other fish. The MT gene of Z. platypus sequenced in the present study can be used as a useful biomarker for heavy metal exposure in the aquatic environment of Korea and other countries where this freshwater fish species represents the ecosystem.

Lee, S. Y. and Y. K. Nam (2016). "Transcriptional responses of metallothionein gene to different stress factors in Pacific abalone (Haliotis discus hannai)." Fish Shellfish Immunol 58: 530-541.

A novel metallothionein (MT) gene from the Pacific abalone H. discus hannai was characterized and its mRNA expression patterns (tissue distribution, developmental expression and differential expression in responsive to various in vivo stimulatory treatments) were examined. Abalone MT shares conserved structural features with previously known gastropod orthologs at both genomic (i.e., tripartite organization) and amino acid (conserved Cys motifs) levels. The 5'-flanking regulatory region of abalone MT gene displayed various transcription factor binding motifs particularly including ones related with metal regulation and stress/immune responses. Tissue distribution and basal expression patterns of MT mRNAs indicated a potential association between ovarian MT expression and sexual maturation. Developmental expression pattern suggested the maternal contribution of MT mRNAs to embryonic and early larval developments. Abalone MT mRNAs could be significantly induced by various heavy metals in different tissues (gill, hepatopancreas, muscle and hemocyte) in a tissue- and/or metal-dependent fashion. In addition, the abalone MT gene was highly modulated in responsive to other non-metal, stimulatory treatments such as immune challenge (LPS, polyI:C and bacterial injections), hypoxia (decrease from normoxia 8 ppm-2 ppm), thermal elevation (increase from 20 degrees C to 30 degrees C), and xenobiotic exposure (250 ppb of 17alpha-ethynylestradiol and 0.25 ppb of 2,3,7,8-tetrachlorodibenzodioxin) where differential expression patterns were toward either up- or down-regulation depending on types of stimulations and tissues examined. Taken together, our results highlight that MT is a multifunctional effector playing in wide criteria of cellular pathways especially associated with development and stress responses in this abalone species.

Lee, W., et al. (1987). "Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionein gene and SV40." Nature 325(6102): 368-372.

Genetic analysis of eukaryotic transcriptional promoters has revealed that protein-coding genes often contain a complex array of cis-control elements consisting of upstream activator sequences and enhancer elements. The metallothionein genes provide a useful example for dissecting the action of multiple interspersed control elements that govern both basal level and regulated expression in animal cells. The human metallothionein (hMTIIA) promoter has been analysed in detail and found to contain no less than five distinct control elements in the 5' flanking regions of

the gene that mediate specificity and regulation of transcription. These different control elements can be functionally subdivided into two categories: basal and induced elements. There are several distinct basal recognition sequences, which include a TATA-box, a GC-box, and at least two basal level enhancer (BLE) sequences, that function like classical enhancer elements. The hMTIIA gene also responds to induction by heavy metals and by steroid hormones through the action of metal regulatory elements (MRE) and glucocorticoid responsive elements (GRE). Here we report the identification of two cellular DNA-binding proteins that interact selectively with sequences governing the basal level expression of hMTIIA. One of these factors is a novel activator protein (AP1) that interacts with sequences in the BLE of hMTIIA and also binds to a site within the 72-base pair (bp) repeats of the simian virus 40 (SV40) enhancer region. The second protein has been purified to homogeneity and shown to be transcription factor Sp1 which recognizes and binds to a single GC-box element within the hMTIIA promoter.

Lee, Y. J., et al. (1996). "Structure and expression of metallothionein gene in ducks." Gene 176(1-2): 85-92.

Metallothionein (MT) cDNAs were cloned and sequenced from two genera of ducks, Muscovy (Cairina muschata) and Tsai ya (Anas platyrhynchos). The two cDNAs show an extremely high sequence homology and contain an open reading frame encoding 63 amino acids. MT mRNA expressions were studied after metal induction using the cloned cDNA as a probe. Cadmium and copper induced MT gene efficiently, whereas zinc showed a markedly less effect. In addition, the MT mRNA accumulations in various developmental stages were also investigated. The result reveals a different pattern of expression from that of mammals. The discrepancy in MT gene between Tsai ya and Muscovy was further explored by examining genomic DNA structures. The duck MT showed three exons and two introns. The most significant variation of the genes occurs at intron II in which Tsai ya MT has 24 bases more than Muscovy MT. Moreover, MT expressions in the hybrids of Muscovy and Tsai ya were investigated using a reverse transcriptase-polymerase chain reaction. Those results demonstrated that parental MT genes are expressed in the hybrids after metal induction.

Lei, Z., et al. (2002). "[Effect of 7,12-dimethylbenz(a)athrancene on immune function in metallothionein gene-knocked-out mice]." Zhonghua Yu Fang Yi Xue Za Zhi 36(6): 398-401.

OBJECTIVE: To study the immunotoxicity induced by 9,10-dimethyl-1,2-benzathrancene (DMBA) in metallothionein gene-knocked-out mice [MT(-/-)] as compared with that in wild-type mice [(MT(+/+)]. METHODS: Female mice were treated with 25 mg/kg and 50 mg/kg of DMBA i.p., respectively and immunized with sheep red blood cells (SRBC) i.v. on the following day and rechallenged by injection of SRBC via footpad s.c. on the fourth day post-immunization. Humoral and cell-mediated immune function was assessed by the number of spleen IgM antibody plaque formation cells (PFC) to SRBC and cell-mediated delayed-type hypersensitivity (DTH) measured by footpad swelling thickness. RESULTS: After treatment with 25 mg/kg DMBA, a decrease in weight of their spleen and thymus and PFC/spleen were observed in MT(-/-) mice, while only decrease in thymus weight of MT(+/+) mice. The humoral function was suppressed by 72% in MT(-/-) mice. No obvious change in cell-mediated immune function was observed both in MT(-/-) and MT(+/+) mice. Both humoral and cell-mediated immune function were suppressed more severe (91%) in MT(-/-) mice treated with 50 mg/kg DMBA than those treated with 25 mg/kg DMBA (72%). DTH was not altered by DMBA in MT(+/+) mice. The weight of their spleen and thymus decreased and humoral immune function suppressed in MT(+/+) mice, but these changes were significantly less severe. No obvious suppression of cell-mediated immune function was observed in MT(+/+) mice. CONCLUSION: Their humoral and cell-mediated immune function was more susceptible to being suppressed by DMBA in MT(-/-) mice, indicating that MT could protect their immune function from damage caused by DMBA.

Leignel, V., et al. (2008). "Metallothionein genes from hydrothermal crabs (Bythograeidae, Decapoda): characterization, sequence analysis, gene expression and comparison with coastal crabs." Comp Biochem Physiol C Toxicol Pharmacol 148(1): 6-13.

Hydrothermal vent conditions can alter DNA and hydrothermal organisms may develop detoxification mechanisms and/or genetic adaptations. Hydrothermal vent animals notably synthesize a high quantity of metallothioneins (MT). Recent studies have revealed that the levels of MT within hydrothermal crustacean tissues are higher than those found in other vent animals. To improve our understanding of the environmental impacts exerted on the vent organisms, we characterized the metallothioneins (cDNA and Mt genes) of several members of the Bythograeidae (Bythograea thermydron, Cyanagraea praedator and Segonzacia mesatlantica) which is the only endemic hydrothermal crab family. In comparison, the isolation of metallothionein cDNA was also carried out in several coastal crab families. The results showed that the hydrothermal crabs possess Mt composed of three exons and two introns presenting conserved splicing signals. The cDNA sequences isolated from distinct crabs showed multiple substitutions. In spite of the unique environmental conditions, the protein sequence analysis revealed no specific amino acid residue for the

MT of the three hydrothermal crabs. However, gene expression analysis performed by real-time PCR based on S. mesatlantica (hydrothermal crab) compared to Pachygrapsus marmoratus (coastal crab) confirmed the higher metallothionein induction in hydrothermal crabs suggested by others authors.

Leone, A. (1986). "Metallothionein gene regulation in Menkes' disease." Horiz Biochem Biophys 8: 207-256.

Metallothioneins are a family of ubiquitous, cysteine rich proteins, whose amino acidic and genomic sequences have been highly conserved during evolution. MT synthesis is induced by heavy metals, glucocorticoids and a bacterial lipopolysaccharide in vivo and in vitro. MT forms stable complexes with heavy metals. One MTIIA gene, four MTI class genes and five pseudogenes have been isolated in humans. The cluster of MT genes is located on chromosome 16. The cloned, transfected genes retain metal inducibility. The first 150 bp of the 5' flanking region of mouse and human MT genes are essential for transcription and metal regulation. Two control regions have been identified. The distal region, between -151 and -78 is essential for efficient transcription and binding of cellular factor(s) which regulates MT gene expression. In Menkes' disease, a lethal X-linked recessive disorder, copper accumulates intracellularly bound to MT. Low doses of copper induce MT synthesis in Menkes' fibroblasts, but not in normal controls. Transfection experiments using the mouse MTI promoter fused to CAT show that the effect of copper in MT transcription is in trans. Menkes' cells are more sensitive to copper than normal controls and respond to copper poisoning by synthesizing two heat-shock like proteins. A mutation affecting copper transport or metabolism is discussed.

Leone, A. and D. H. Hamer (1987). "Abnormal copper metabolism and regulation of metallothionein gene expression in Menkes' disease." Experientia Suppl 52: 477-480.

Menkes' kinky hair disease, a lethal X-linked recessive trait, is characterized by abnormal copper accumulation in several non-hepatic tissues. The level of many copper enzymes is severely reduced, leading to damage of the connective and nervous tissues of the patients. Cultured skin fibroblasts from Menkes' patients retain more copper then normal controls, and the excess metal is bound to metallothionein. Low doses of copper in the media induce MT gene transcription in Menkes' but not in normal cells. Transfection experiments using a plasmid containing the mouse MT-I promoter fused to the enzyme chloramphenicol acetyl transferase show that the activation of the mMTI promoter is in trans. Two other effects are observed in Menkes' cells: (a) two heat-shock like proteins are synthesized in response to low doses of copper in the growth medium, and (b) Menkes' cells are more sensitive then normal fibroblasts to copper toxicity. Our interpretation of these results supports a model for a defect in one or more steps in copper metabolism or transport.

Lieb, B. (2003). "A new metallothionein gene from the giant keyhole limpet Megathura crenulata." Comp Biochem Physiol C Toxicol Pharmacol 134(1): 131-137.

Metallothioneins (MTs) are small soluble proteins ubiquitously expressed in animals and plants. Different isoforms are present in deuterostomes and protostomes. They do not differ greatly in primary structure, but are clearly distinguishable. Here, I present the gene and the complete cDNA of a novel MT from the mollusk Megathura crenulata. This protein is closely related to the Cu-inducible MTs of the vineyard snail Helix pomatia, but has also some minor sequence features typical of Cd-inducible isoforms of H. pomatia and other molluscs. Overall, the deduced primary structure is similar to the known molluscan MTs, but in addition possesses an insertion of 5 amino acids not found in any other molluscan MTs, protostomic or deuterostomic MTs. In addition, a pentapeptide insertion, characteristic of mammalian MT-3 is present but it lacks the functional tetrapeptide CPCP within the beta-region of those MT-3 proteins that are known to suppress neuronal growth processes. The M. crenulata MT is a novel form of MT in comparison to all other known MTs. Possible functional aspects for this new MT are discussed.

Lieberman, H. B., et al. (1985). "Human metallothionein-II processed gene is located in region p11----q21 of chromosome 4." Cytogenet Cell Genet 39(2): 109-115.

Metallothionein (MT) genes comprise a multigene family encoding low-molecular-weight, heavy-metal-binding proteins. We have mapped a human MT-II processed gene to chromosome 4, using Southern blotting in combination with a human X mouse hybrid clone panel containing defined subsets of human chromosomes. We have further localized this gene to region p11----q21, using in situ hybridization.

Lieberman, M. W., et al. (1983). "Ultraviolet radiation-induced metallothionein-I gene activation is associated with extensive DNA demethylation." Cell 35(1): 207-214.

Ultraviolet irradiation (UV) of cadmium-sensitive S49 mouse cells induces a large increase in cadmium-resistant variants. About 30%-40% of these variants make metallothionein (MT)-I mRNA while S49 cells do not. S49 cells contain two copies of the MT-I gene; both alleles are heavily methylated but can be conveniently distinguished by the methylation status of a single Hpa II site. In lines expressing MT-I, one allele becomes completely demethylated at all methylation-sensitive restriction sites examined over at

least a 2.5 kb region spanning the MT-I gene. Activation of a quiescent gene by UV has implications for understanding the initiation of carcinogenesis.

Lim, D., et al. (2009). "Silencing the Metallothionein-2A gene inhibits cell cycle progression from G1- to S-phase involving ATM and cdc25A signaling in breast cancer cells." Cancer Lett 276(1): 109-117.

Metallothioneins (MTs) are a group of metal-binding proteins involved in cell proliferation, differentiation and apoptosis. The MT-2A isoform is generally the most abundant isoform among the 10 known functional MT genes. In the present study, we observed that down-regulation of the MT-2A gene in MCF-7 cells via siRNA-mediated silencing inhibited cell growth by inducing cell cycle arrest in G1-phase (G1-arrest) and a marginal increase in cells in sub-G1-phase. Scanning electron microscopic examination of the cells with silenced expression of MT-2A (siMT-2A cells) revealed essentially normal cell morphology with presence of scattered apoptotic cells. To elucidate the underlying molecular mechanism, we examined the expression of cell cycle related genes in MT-2A-silenced cells and found a higher expression of the ataxia telangiectasia mutated (ATM) gene concomitant with a lower expression of the cdc25A gene. These data suggest that MT-2A could plausibly modulate cell cycle progression from G1- to S-phase via the ATM/Chk2/cdc25A pathway.

Lin, C. H., et al. (2004). "Cloning and characterization of metallothionein gene in ayu Plecoglossus altivelis." Aquat Toxicol 66(2): 111-124.

Metallothionein (MT) has been used widely as a potential molecular marker to detect the deleterious effects of heavy metals in aquatic ecosystem. Here we exposed ayu, Plecoglossus altivelis, to zinc (Zn) and tested the distribution as well as the induction of MT in various tissues such as liver, kidney, intestine and stomach. MT induction was significant in liver tissue, followed by kidney and intestine, whereas no induction was detected in stomach. The gene encoding ayu MT was successfully cloned and characterized. Complete nucleotide sequencing and analysis of the 4.5 kb DNA fragment containing the ayu MT gene revealed that the gene has three exons interrupted by two introns, a 5'-flanking region of about 2.5 kb and about 1.6 kb of 3'-flanking region. In grouper heart and kidney cells, the 2.5 kb promoter containing eight metal responsive elements (MREs), two hepatic nuclear factor 5 responsive elements (HNF5REs) and one cAMP responsive element (CRE) had the highest reporter activity.

Lin, K. A., et al. (2005). "Alkaline induces metallothionein gene expression and potentiates cell proliferation in Chinese hamster ovary cells." J Cell Physiol 205(3): 428-436.

Metallothionein (MT) gene expression is increased in cadmium resistant Chinese hamster ovary cells (CHO Cd(R)) upon medium (regular or serum-free) change during culturing. Among the major components of the medium, NaHCO3 was found to be able to induce MT gene expression in a dose- and time-dependent manner. The same effect was observed with other alkaline solutions, such as HEPES and NaOH. Using MT promoter-luciferase reporter gene constructs, we found that the presence of metal response elements (MREs) in the promoter region is necessary for NaHCO3-induced MT gene transcription. This finding is further supported by the observation that the binding activity between the metal-responsive transcription factor 1 (MTF-1) and the MRE were increased after NaHCO3 treatment. Following NaHCO3 treatment, an increase in cell proliferation was observed in CdR cells but not in the parental CHO K1 cells that do not express MT transcripts due to MT gene methylation. Using synchronized cells, an increase in cell proliferation was observed 9 h after NaHCO3 addition. Notably, proliferation of CHO K1 cells was increased when transfected with an MT gene. The effect of MT on cell growth was affirmed by treating CHO K1 cells with 5-azacytidine (Aza) to demethylate the MT gene. Proliferation increased in Aza-treated CHO K1 cells after NaHCO3 treatment. These results demonstrate that NaHCO3 stimulates MT gene expression and causes an enhancement of cell proliferation in CHO cells.

Liu, H. L., et al. (2020). "Effects of Vitamin D Receptor, Metallothionein 1A, and 2A Gene Polymorphisms on Toxicity of the Peripheral Nervous System in Chronically Lead-Exposed Workers." Int J Environ Res Public Health 17(8).

Chronic exposure to lead is neurotoxic to the human peripheral sensory system. Variant vitamin D receptor (VDR) genes and polymorphisms of metallothioneins (MTs) are associated with different outcomes following lead toxicity. However, no evidence of a relationship between lead neurotoxicity and polymorphisms has previously been presented. In this study, we investigated the relationship between the polymorphisms of VDR, MT1A, and MT2A genes and lead toxicity following chronic occupational lead exposure. We measured vibration perception thresholds (VPT) and current perception thresholds (CPT) in 181 workers annually for five years. The outcome variables were correlated to the subject's index of long-term lead exposure. Polymorphisms of VDR, MT1A, and MT2A were defined. The potential confounders, including age, sex, height, smoking, alcohol consumption, and working life span, were also collected and analyzed using linear regression. The regression coefficients of some gene polymorphisms were at least 20 times larger than regression coefficients of time-weighted index of cumulative blood lead (TWICL) measures. All

regression coefficients of TWICL increased slightly. MT1A rs11640851 (AA/CC) was associated with a statistically significant difference in all neurological outcomes except hand and foot VPT. MT1A rs8052394 was associated with statistically significant differences in hand and foot CPT 2000 Hz. In MT2A rs10636, those with the C allele showed a greater effect on hand CPT than those with the G allele. Among the VDR gene polymorphisms, the Apa rs7975232 (CC/AA) single nucleotide polymorphism was associated with the greatest difference in hand CPT. MT2A rs28366003 appeared to have a neural protective effect, whereas Apa (rs7975232) of VDR and MT2A rs10636 increased the neurotoxicity as measured by CPT in the hands. MT1A rs8052394 had a protective effect on large myelinated nerves. MT1A rs11640851 was associated with susceptibility to neurotoxicity.

Liu, J., et al. (2006). "Differential effects between maotai and ethanol on hepatic gene expression in mice: Possible role of metallothionein and heme oxygenase-1 induction by maotai." Exp Biol Med (Maywood) 231(9): 1535-1541.

Alcohol is a risk factor for liver fibrosis and hepatocellular carcinoma. On the other hand, light alcoholic beverage consumption is believed to be beneficial because of the effects of both alcohol and nonalcoholic components of the beverage. Maotai is a commonly consumed beverage in China containing 53% alcohol. Epidemiological and experimental studies show that Maotai is less toxic to the liver than ethanol alone. To examine the differential effects of Maotai and ethanol, a low dose of Maotai or an equal amount of ethanol (53%, v/v in water, 5 ml/kg) were given to male mice daily for 1 week, and hepatic RNA was extracted for microarray analysis. Approximately 10% of genes on the liver-selective custom array (588 genes) were altered following Maotai or ethanol administration, but Maotai treated livers had fewer alterations compared with ethanol alone. Real-time reverse transcription-polymerase chain reaction confirmed and extended microarray results on selected genes. An induction of metallothionein and heme oxygenase-1 occurred with Maotai, which could not be explained by alcohol consumption alone, whereas the attenuation of ethanol responsive genes such as quinone dehydrogenase, DNA-ligase 1, IGFBP1, and IL-1beta suggests less liver injury occurred with Maotai. The expression of genes related to liver fibrosis, such as cytokeratin-18, was slightly increased by the high dose of ethanol, but was unchanged in the Maotai group. In summary, gene expression analysis indicates that Maotai induces a different response than ethanol alone. The dramatic induction of metallothionein and heme oxygenase-1 with Maotai could be important adaptive responses to reduce alcoholic liver injury.

Liu, J., et al. (2002). "Acute cadmium exposure induces stress-related gene expression in wild-type and metallothionein-I/II-null mice." Free Radic Biol Med 32(6): 525-535.

This study examined the effect of acute cadmium on stress-related gene expression and free radical production in wild-type and metallothionein-I/II-null (MT-null) mice. Atlas Toxicology arrays showed that acute cadmium (40 micromol/kg as CdCl(2), ip for 3 h) markedly increased the expression of genes encoding heat-shock proteins, heme oxygenase-1, and genes in response to DNA damage/repair. The expression of genes encoding cytochrome P450 enzymes, UDP-glucuronosyltransferases, Mn-superoxide dismutase, and catalase was suppressed by cadmium. MT-null mice were more sensitive than wild-type mice to cadmium-induced, stress-related gene expression, in accord with greater activation of transcription factor AP-1 and phosphorylated JNK and ERK. To evaluate free radical production, mice were simultaneously given the spin trap agent, N-tert-butyl-alpha-phenylnitrone (PBN, 250 mg in DMSO/kg, ip) with cadmium, and livers were removed 30 min later for PBN-trapped radical extraction with chloroform:methanol (2:1), and detected with electron spin resonance (ESR). Cadmium treatment caused detectable ESR signals for PBN adducts as well as lipid peroxidation in the liver similarly in both wild-type and MT-null mice. Thus, the mechanism of acute cadmium toxicity involves multiple facets including oxidative damage and aberrant gene expression, and absence of MT exacerbates Cd-induced aberrant gene expression.

Liu, J., et al. (2015). "Copper-induced hydrogen peroxide upregulation of a metallothionein gene, OsMT2c, from Oryza sativa L. confers copper tolerance in Arabidopsis thaliana." J Hazard Mater 294: 99-108.

Metallothioneins (MTs) are low-molecular-weight, cysteine-rich metal-binding proteins found in numerous genera and species, but their functions in abiotic stress tolerance remain unclear. Here, a MT gene from Oryza sativa, OsMT2c, was isolated and characterized, encoding a type 2 MT, and observed expression in the roots, leaf sheathes, and leaves, but only weak expression in seeds. OsMT2c was upregulated by copper (Cu) and hydrogen peroxide (H2O2) treatments. Excessive Cu elicited a rapid and sustained production and release of H2O2 in rice, and exogenous H2O2 scavengers N,N'-dimethylthiourea (DMTU) and ascorbic acid (Asc) decreased H2O2 production and OsMT2c expression. Furthermore, the expression of OsMT2c increased in the osapx2 mutant in which the H2O2 levels were higher than in wild-type (WT) plants. These results showed that Cu increased MT2c expression through the production and

accumulation of Cu-induced H2O2 in O. sativa. In addition, the transgenic OsMT2c-overexpressing Arabidopsis displayed improved tolerance to Cu stress and exhibited increased reactive oxygen species (ROS) scavenging ability compared to WT and empty-vector (Ev) seedlings.

Liu, X. D. and D. J. Thiele (1996). "Oxidative stress induced heat shock factor phosphorylation and HSF-dependent activation of yeast metallothionein gene transcription." Genes Dev 10(5): 592-603.

Metallothioneins (MTs) are a class of low-molecular-weight, cysteine- rich metal-binding proteins that function in metal detoxification and oxidative stress protection. We demonstrate that transcription of the Saccharomyces cerevisiae MT gene CUP1 is strongly activated by the superoxide anion generator menadione. This activation is exacerbated in a strain lacking the gene encoding Co, Zn superoxide dismutase (SOD1). CUP1 transcriptional activation by oxidative stress is dependent on a functional CUP1 promoter heat shock element (HSE) and the carboxy-terminal trans-activation domain of heat shock transcription factor (HSF). Furthermore, protection against oxidative stress conferred by CUP1 in a (sod1)delta strain requires HSF-mediated CUP1 transcription. Although in response to heat, HSF-mediated CUP1 transcription and HSF phosphorylation are transient, both CUP1 gene expression and HSF phosphorylation are sustained in response to oxidative stress. Moreover, the patterns of tryptic phosphopeptides resolved from HSF derived from cells subjected to heat shock or oxidative stress are distinct. These results demonstrate that transcription of the S. cerevisiae metallothionein gene under conditions of oxidative stress is mediated by HSF and that in response to distinct activation stimuli, HSF is differentially phosphorylated in a manner that parallels metallothionein gene transcription.

Liu, X. D. and D. J. Thiele (1997). "Yeast metallothionein gene expression in response to metals and oxidative stress." Methods 11(3): 289-299.

Metals and oxygen are chemically linked in biological systems. Metals and oxygen play important roles in enzymatic reactions, metabolism, and signal transduction; however, metals and oxygen react to form highly toxic oxygen-derived free radical species. In this review we focus on the use of yeast cells, as unicellular eukaryotic model systems, to conduct studies aimed at understanding fundamental mechanisms for the sensation and protective responses to toxic metals and oxygen-derived radicals via the activation of yeast metallothionein gene expression.

Liu, Z., et al. (2016). "Cloning and characterization of metallothionein gene (HcMT) from Halostachys caspica and its expression in E. coli." Gene 585(2): 221-227.

Halostachys caspica is a short shrub distributed in the semi-arid and saline-alkali area, which evolved various mechanisms for modulating salt and metal level. In the present study, a Type 2 metallothionein (HcMT) gene was cloned from the salt induced suppression subtractive hybridization (SSH) cDNA library of H.caspica. Quantitative real time PCR (qRT-PCR) analysis indicated that HcMT gene was up-regulated under the stress of Cu(2+), Zn(2+) and Cd(2+), and the tolerance of E. coli strain harboring with the recombinant HcMT (pET-32a-HcMT) to Cu(2+), Zn(2+) and Cd(2+) was enhanced compared to strain with control vector (pET-32a). Moreover, the purified TrxA-HcMT fusion protein from E. coli cells grown in the presence of 0.3mM CuSO4, 0.3mM ZnSO4, or 0.1mM CdCl2 could bind more metal ions than TrxA alone. The predicted 3D structure showed that HcMT could form a single metal-thiolate cluster, which confers the ability to bind five divalent metal ions through fourteen cysteine residues. These data indicate that HcMT may be involved in processes of metal tolerance in H. caspica and could be employed as a potential candidate for heavy metal phytoremediation.

Loney, K. D., et al. (2003). "Strain-specific brain metallothionein II (MT-II) gene expression, its ethanol responsiveness, and association with ethanol preference in mice." Alcohol Clin Exp Res 27(3): 388-395.

BACKGROUND: Metallothioneins (MTs) are ubiquitously expressed intracellular proteins that bind heavy metals such as zinc, copper, and cadmium. Although their specific function has yet to be discovered, they are known to regulate the metabolism of these metals as well as respond to cellular stress agents, particularly oxidants. METHODS: Brain RNA from experimental (8 g/kg 25% ethanol injection) and control (saline injection) mice from four strains (A/J, BALB/cJ, C57BL/6J, DBA/2J) that are known to differ with respect to ethanol preference was used in differential displays. This report includes molecular results on one gene (MT-II) identified. RESULTS: Our results on differential displays suggest that a proportion of genes are differentially expressed across pair-wise strain comparisons. We identified MT-II as a strain-specific and ethanol-responsive gene. The level of MT-II messenger RNA (mRNA) in control mice of A/J, BALB/cJ, C57BL/6J, and DBA/2J strains was variable (0.50, 0.51, 0.90, and 0.14 times G3PDH expression, respectively). The degree of up-regulation in experimental mice was also somewhat variable among strains, ranging from 2.5 to 3.2 times expression over the matched controls. Experiments indicate that the promoter and genomic organization of the MT-II gene is identical in sequence for all four strains, and methylation studies revealed that the MT-II promoter region is unmethylated in the brains of these mice. Interestingly, MT-II expression in control mice

demonstrated a positive correlation with the ethanol preference phenotype. CONCLUSION: An increase in MT-II mRNA levels after injection of ethanol is attributed to the antioxidant properties of MT-II. The differential mRNA levels of this gene among four strains are not accounted for by the genomic organization, DNA sequence, or methylation status of this gene. Furthermore, the observed correlation between MT-II mRNA levels and ethanol preference raises an interesting hypothesis about the possible role of MT-II in ethanol effects and preference in mice.

Loney, K. D., et al. (2006). "Analysis of metallothionein brain gene expression in relation to ethanol preference in mice using cosegregation and gene knockouts." Alcohol Clin Exp Res 30(1): 15-25.

BACKGROUND: Metallothioneins (MTs) are ubiquitously expressed intracellular proteins that bind heavy metals and are involved in cytoprotection against several types of stress agents including chemicals, hormones, and oxidants. We have previously reported 1 isoform, MT-II, as a possible candidate gene for ethanol (EtOH) preference (EP) determination in mice. METHODS: Semiquantitative RT-PCR was used to determine brain mRNA levels of MT-I and MT-III in 4 inbred mouse strains with variable EP. Following this, cosegregation of MT-II brain expression with EP was analyzed in F2 mice from 2 intercrosses (C57BL/6J x BALB/cJ and C57BL/6J x DBA/2J). Studies on MT-I/MT-II knockout (KO) mice were also undertaken to further explore this relationship. RESULTS: Our results suggest that MT-I is responsive to EtOH, with no evidence of basal-level differences between strains. Conversely, MT-III shows no EtOH response, yet indicates a possible strain-specific feature with C57BL/6J having the lowest levels of brain MT-III. Metallothionein-II expression cosegregates with EP in F2 mice from a C57BL/6J (preferring) and DBA/2J (avoiding) intercross. Although F2 mice from a cross with C57BL/6J and BALB/cJ (avoiding) strains follow a similar pattern, the results are not statistically significant. Metallothionein-I/MT-II knockout (MT-KO) mice appear to have smaller litter sizes as well as higher weight compared with controls (129S1/SvImJ) and also show a slight increase in EP. CONCLUSIONS: Metallothionein-II remains the primary candidate of the mouse MT gene family for involvement in EP. Its effect on EP appears to be dependent on the genetic background. Such conclusions are based on results from C57BL/6J, BALB/cJ, DBA/2J, and 129 inbred mouse strains. Evidence also points to shared neural pathways involved in weight gain and obesity. The complex interactions between MT-II, EP, and weight gain/obesity remain to be studied.

Low, M. J., et al. (1984). "High plasma levels of immunoreactive somatostatin in transgenic mice expressing a metallothionein-somatostatin fusion gene." Trans Assoc Am Physicians 97: 205-209.

To test the hypothesis that processing of pre-prosomatostatin (pre-proSS) can be accomplished by cells that do not normally synthesize the precursor, we have introduced the rat pre-proSS gene under control of the mouse metallothionein promoter into the germ line of mice. Four of the 11 resultant transgenic mice had markedly elevated plasma levels of somatostatin-like immunoreactivity (SLI); however, their growth was identical to control littermates. Liver contained 263 +/- 89 pg of SLI/mg of protein and kidney had 152 +/- 19 pg/mg. Gel filtration chromatography of tissue extracts resolved one major 6000-dalton peak of SLI and three minor peaks of 8500, 3000, and 1600 daltons. The latter two corresponded in elution position to synthetic somatostatin-28 (S-28) and somatostatin-14 (S-14). Almost all of the plasma SLI corresponded in size to the 6000-dalton peptide. These findings indicate that a metallothionein-somatostatin fusion gene was successfully integrated into the mouse genome and was expressed in tissues that do not normally synthesize pre-proSS. Pre-proSS was processed to S-28 and S-14 but atypical processing to a 6000-dalton peptide also occurred.

Low, M. J., et al. (1985). "Tissue-specific posttranslational processing of pre-prosomatostatin encoded by a metallothionein-somatostatin fusion gene in transgenic mice." Cell 41(1): 211-219.

The somatostatins are neuropeptides of 14 and 28 amino acids that inhibit the release of growth hormone and other hypophyseal and gastrointestinal peptides. These neuropeptides are cleaved posttranslationally from a common precursor, pre-prosomatostatin. We report here the production and processing of pre-prosomatostatin by transgenic mice carrying a metallothionein-somatostatin fusion gene. The most active site of somatostatin production, as determined by hormone concentrations in the tissues, is the anterior pituitary, a tissue that does not normally synthesize somatostatin-like peptides. Anterior pituitary processed pre-prosomatostatin almost exclusively to the two biologically active peptides, somatostatin-14 and somatostatin-28, whereas the liver and kidney synthesized much smaller quantities of predominantly a 6000 dalton somatostatin-like peptide. The growth of the transgenic mice was normal despite high plasma levels of the somatostatin-like peptides. These studies indicate that proteases which cleave prosomatostatin to somatostatin-28 and somatostatin-14 are not specific to tissues that normally express somatostatin.

Low, M. J., et al. (1986). "Somatostatin is targeted to the regulated secretory pathway of gonadotrophs in transgenic mice expressing a metallothionein-somatostatin gene." J Biol Chem 261(34): 16260-16263.

The pituitaries of transgenic mice that express a metallothionein-somatostatin fusion gene contain high concentrations of somatostatin-14 exclusively in the gonadotrophic cells. The purpose of this study was to determine whether somatostatin expressed from the foreign fusion gene enters the normal secretory pathway within these cells. Immuno-gold labeling of serial thin sections localized somatostatin to the secretory granules of gonadotropin-producing cells. The gonadotroph-specific hypophysiotropic factor, luteinizing hormone-releasing hormone caused a dose-dependent secretion of somatostatin when applied to primary pituitary cultures from these mice. Growth hormone-releasing hormone, thyrotropin-releasing hormone, corticotropin releasing factor, and dopamine did not affect somatostatin secretion. These experiments demonstrate that a neurosecretory peptide encoded by a foreign gene can enter the regulated secretory pathway of pituitary cells from transgenic mice.

Lu, D. D., et al. (2003). "The relationship between metallothionein-1F (MT1F) gene and hepatocellular carcinoma." Yale J Biol Med 76(2): 55-62.

To investigate the expression of MT1F gene in hepatocellular carcinoma tissue and the growth suppression effect of exogenous introduction of MT1F gene on liver cell line HepG2 and to explore the potential application of MT1F gene in gene therapy of tumor. Eukaryotic expression vector of pCMV-MT1F plasmid was introduced into HepG2 line which expressed no MT1F protein originally with lipofectamine transfection method. The cell growth curve, soft agar colony formation rate and tumorigenicity in SCID mice were examined to demonstrate the growth suppression effect of exogenous MT1F gene on HepG2 cell line. The MT1F mRNA and MT1F protein were also detected in 60 pairs of surgical specimens of hepatocellular carcinoma by in situ hybridization and immunohistochemistry. The transfected HepG2 cell line grew more slowly than control HepG2 as shown by cell growth curves, the soft agar colony formation rate (3.8 percent vs. 7.4 percent, p <.01) and the average growth rate of tumor in SCID mice (30.9 +/- 6.9 vs. 70.3 +/- 5.6, p <.01). The expression level of MT1F mRNA and protein significantly increased in paracancerous tissue, normal tissue than in cancer tissues (75 percent, 70 percent vs. 16.7 percent by ISH and 66.7 percent, 60 percent vs. 10 percent by IHC, p <.01). Exogenous MT1F gene shows the strong effect of growth inhibition on HepG2 cell line. In the liver cancer tissue, MT1F shows down-regulated expression that supports the inhibited function of MT1F in cancer growth and suggests MT1F may have an important role in gene therapy of hepatocellular carcinoma.

Lu, J., et al. (2001). "Metallothionein gene expression in peripheral lymphocytes from cadmium-exposed workers." Cell Stress Chaperones 6(2): 97-104.

Metallothionein (MT) plays an important role in the detoxification of cadmium. To investigate the usefulness of MT gene expression in peripheral blood lymphocytes (PBLs) as a biomarker of cadmium exposure and susceptibility, reverse transcriptase-polymerase chain reaction was used to measure the MT gene expression in PBLs from cadmium-exposed workers. Both basal and induced MT expressions were found to increase with increased blood cadmium (BCd) and urinary cadmium (UCd) levels. Both basal and induced MT expression levels were significantly correlated with the logarithm of BCd and the logarithm of UCd levels. The dose-response relationship between internal dose of cadmium and MT expression suggested the validity of MT expression in PBLs as a biomarker of cadmium exposure. In vitro induced MT expression level in PBLs was found to be inversely related to the level of renal dysfunction indicator, urinary N-acetyl-beta-D-glucosaminidase (UNAG). The latter finding indicates that MT expression in PBLs may be a useful biomarker of susceptibility to renal toxicity of cadmium.

Lu, J., et al. (2005). "Metallothionein gene expression in peripheral lymphocytes and renal dysfunction in a population environmentally exposed to cadmium." Toxicol Appl Pharmacol 206(2): 150-156.

In order to study the validity of metallothionein (MT) gene expression in peripheral blood lymphocytes (PBLs) as a biomarker of cadmium exposure and susceptibility to renal dysfunction, MT mRNA levels were measured using reverse transcription polymerase chain reaction (RT-PCR) in PBLs from residents living in a cadmium-contaminated area. MT mRNA levels were found to increase with the increase of blood cadmium (BCd) and urinary cadmium (UCd) levels. Basal MT mRNA levels were significantly correlated with the logarithm of BCd levels and the logarithm of UCd levels confirming that MT expression in PBLs is a biomarker of cadmium exposure and internal dose. An inverse relationship was observed between in vitro induced MT-mRNA level in PBLs and urinary N-acetyl-beta-d-glucosaminidase (UNAG) suggesting that MT gene expression in PBLs may be used as a biomarker of susceptibility to renal toxicity of cadmium.

Ma, C., et al. (2007). "Metallothionein I and II gene knock-out mice exhibit reduced tolerance to 24-h sodium lauryl sulphate patch testing." Clin Exp Dermatol 32(4): 417-422.

BACKGROUND: Metallothioneins (MTs) are a group of proteins widely distributed in tissues regulating metal metabolism, scavenging free radicals, and taking part in immunological reactions. Knockout mice for MT genes I and II (MT(-/-)) exhibit reduced

tolerance to ultraviolet B injury in vivo. Upregulation of MT proteins can be found at positive allergy patch-test sites; however, the role of MTs in skin irritation has not been investigated. AIM: To evaluate the role of MT genes in sodium lauryl sulphate (SLS)-induced skin irritation. SLS is a well-known model irritant in the study of experimental irritant contact dermatitis. METHODS: Skin irritation was induced in mice by applying closed-patch testing of 2.5%, 5%, 7.5% and 10% SLS in distilled water on the right dorsal skin of MT(-/-) mice for 24 h. Skin irritation was evaluated visually and by the number of infiltrated inflammatory cells in SLS-irritated skin. Homozygous wild-type mice with intact MT genes (MT(+/+)) tested at the same time served as controls. RESULTS: MT(-/-) mice showed a much higher degree of skin inflammation than did MT(+/+) mice. Numbers of infiltrated inflammatory cells were 312.8 +/- 50.9 vs. 136.2 +/- 13.1 for 2.5%, 430.2 +/- 49.3 vs. 242.6 +/- 28.6 for 5%, 540.2 +/- 28.4 vs. 437.6 +/- 22.2 for 7.5%, and 690.6 +/- 31.0 vs. 559.0 +/- 37.8 for 10% SLS in MT(-/-) and MT(+/+) mice, respectively ($P < 0.05$, Mann-Whitney U test). CONCLUSIONS: These results clearly suggest that the MTI and MTII genes may play an important protective role in SLS irritation. It would be valuable to study whether topical MTs can prevent or treat skin irritation.

MacArthur, C. A. and M. W. Lieberman (1987). "Different types of hypersensitive sites in the mouse metallothionein gene region." J Biol Chem 262(5): 2161-2165.

We have examined the chromatin structure of the metallothionein (MT) gene region in MT- S49 mouse lymphoma cells and in derivatives which express MT-I alone, MT-II alone, or both genes. In all lines, these genes are contained in a 16-kilobase pair region between two DNase I sensitive sites: one site located 5.3 kilobase pairs 5' of MT-II (the 5' gene) is present in naked DNA and retained in the chromatin of all lines; the other site located 3.1 kilobase pairs 3' of MT-I is hypersensitive. Hypersensitivity at three other sites is dependent on the expression of MT genes. Two sites 5' of MT-II disappear, and a site 3' of MT-I appears regardless of which gene is activated. The fact that these sites respond when either gene is activated suggests that the regulation of the two genes is interdependent and that the region undergoes a general change in conformation with MT activation. In addition, a single site in the 5' region of MT-II becomes hypersensitive with activation of the gene and may be related directly to expression.

Maguire, K. A., et al. (1987). "Accurate transcription of mouse metallothionein-I gene in a fractionated nuclear extract from a rat hepatoma." J Biol Chem 262(9): 3932-3935.

Nuclear extract from Morris hepatoma 3924A was fractionated by DEAE-Sephadex chromatography. The fraction eluting with 300 mM (NH4)2SO4 (DE-C) was used for transcribing cloned mouse metallothionein-I (MT-I) gene in a run-off assay. This fraction contained the majority of RNA polymerase II as well as the transcription factor(s). Accuracy of MT-I DNA transcription was confirmed by S1 nuclease mapping. Low concentrations (1 microgram/ml) of alpha-amanitin inhibited the reaction, indicating that RNA polymerase II directed the transcription. Unfractionated nuclear extracts from the hepatoma or a rat mammary adenocarcinoma as well as whole cell extract obtained from the mammary tumor also transcribed MT-I gene. The extent of transcriptional activity was in the following order: hepatoma nuclear fraction DE-C greater than whole cell extract derived from rat mammary adenocarcinoma cells greater than nuclear extract derived from rat hepatoma or rat mammary adenocarcinoma cells. These studies have demonstrated that a fractionated nuclear extract obtained from a tissue supports efficient and accurate RNA polymerase II-mediated transcription of MT-I DNA.

Mahmood, K., et al. (2009). "Response of metallothionein gene-1 to laboratory exposure to heavy metals and thermal stress in the freshwater prawn Macrobrachium rosenbergii." J Hazard Mater 167(1-3): 523-530.

Metallothioneins, metal-inducible proteins, are being characterized from different organisms and shown as potential biomarkers of exposure to pollution by certain heavy metals. Here we report the identification of a new metallothionein cDNA (433bp) from the shrimp Macrobrachium rosenbergii, putatively encoding a 61 residue polypeptide. Tissue specific analysis indicated that Mar-MT-I (M. rosenbergii Metallothionein Gene-1) is expressed with the highest levels in the hepatopancreas and lowest in the thoracic ganglia, and none in the gills or muscles. In addition, our data showed that Mar-MT-I is differentially regulated in the hepatopancreas by certain heavy metals and thermal stress: Cd and Cu produce somewhat similar expression profile patterns, Zn has a reductional effect and thermal stress alone entirely stops its expression. These results show that Mar-MT-I mRNA levels can potentially be used as biomarkers for Cd, Cu or Zn pollution individually. However, in the case of combined metal treatment, different combinations of these metals have quite different effect on Mar-MT-I expression. Therefore, factors of such differential behaviors should be kept as a priority for further biomonitoring studies.

Maiti, I. B., et al. (1989). "Inheritance and expression of the mouse metallothionein gene in tobacco: impact on cd tolerance and tissue cd distribution in seedlings." Plant Physiol 91(3): 1020-1024.

Genetically engineered seedlings obtained from self-fertilized transgenic tobacco (Nicotiana tabacum) contained and expressed the mouse metallothionein and kanamycin resistance marker genes and were more tolerant to cadmium stress than untransformed controls. Cadmium accumulation in leaves of transgenic seedlings exposed to a low, field-like Cd concentration (0.02 micromolar) was about 20% lower than that in untransformed controls. Genetic analysis of R1 and R2 progeny showed inheritance of the marker gene to be as a dominant Mendelian trait. These results suggest the possibility of developing transgenic plants with modified tolerance to heavy metal stress and food crops having lower Cd content.

Majumder, S., et al. (1999). "Silencing of metallothionein-I gene in mouse lymphosarcoma cells by methylation." Oncogene 18(46): 6287-6295.

Metallothionein-I (MT-I) gene is silenced by methylation of CpG islands in mouse lymphosarcoma P1798 cells but not in the thymus, the cell type from which the tumor was derived. Bisulfite genomic sequencing revealed that all 21 CpG dinucleotides present within -216 bp to +1 bp with respect to transcription start site are methylated in the tumor cell line, but none is methylated in the thymus. The lymphosarcoma cells induced MT-I in response to heavy metals only after demethylation with 5-azacytidine (5-AsaC). The electrophoretic mobility shift assay using specific oligonucleotide probes showed that the key transcription factors regulating MT-I gene (e.g., MTF-1, Sp 1 and MLTF/USF) are active in P1798 cells. In vivo footprinting of the proximal promoter region showed that none of the metal regulatory elements (MREs) or MLTF/USF are occupied in response to heavy metals. Demethylation of the lymphosarcoma cells with 5-AzaC resulted in constitutive footprinting at MLTF/ARE, and zinc-inducible footprinting at MRE-c, MRE-d and MRE-e sites. Demethylation of just 10-20% of the CpG islands was sufficient to render the gene inducible by cadmium or zinc. The MT-I induction persisted in the cancer cells for several generations even after withdrawal of 5-AzaC from the culture medium.

Majumder, S., et al. (2003). "Chromium(VI) down-regulates heavy metal-induced metallothionein gene transcription by modifying transactivation potential of the key transcription factor, metal-responsive transcription factor 1." J Biol Chem 278(28): 26216-26226.

The robust induction of metallothionein-I and II (MT-I and MT-II) genes by several heavy metals such as zinc and cadmium requires the specific transcription factor metal-responsive transcription factor 1 (MTF1). Chromium (VI), a major environmental carcinogen, not only failed to activate these genes but also inhibited their induction by Zn2+ or Cd2+. The heavy metal-induced expression of another MTF1 target gene, zinc transporter 1 (ZnT-1), was also down-regulated by Cr6+. By contrast, the expression of two MTF1-independent Cd2+-inducible genes, heme oxygenase 1 (HO-1) and HSP-70, was not sensitive to Cr6+. Cr6+ did not also affect the expression of housekeeping genes such as GAPDH or beta-actin. Stable cell lines overexpressing variable levels of MTF1, the key transactivator of the MT genes, demonstrated differential resistance toward the inhibitory effect of Cr6+, indicating MTF1 as a target of chromium toxicity. The basal and inducible binding of MTF1 to metal response elements was not affected by treatment of cells with Cr6+. Transient transfection studies showed that the ability of MTF1 to transactivate the MT-I promoter was significantly compromised by Cr6+. The fusion protein consisting of a Gal-4 DNA binding domain and one or more of the three transactivation domains of MTF1, namely the acidic domain, proline-rich domain, and serine-threonine rich domain, activated the GAL-4-driven luciferase gene to different degrees, but all were sensitive to Cr6+. MTF1 null cells were prone to apoptosis after exposure to Zn2+ or Cd2+ that was augmented in presence Cr6+, whereas the onset of apoptosis was significantly delayed in cells overexpressing MTF1.

Majumder, S., et al. (2006). "Epigenetic regulation of metallothionein-i gene expression: differential regulation of methylated and unmethylated promoters by DNA methyltransferases and methyl CpG binding proteins." J Cell Biochem 97(6): 1300-1316.

Metallothioneins (MTs) are a group of cysteine-rich stress response proteins that scavenge reactive oxygen species and heavy metals. Recently, we have shown that MT-I promoter is methylated and suppressed in some solid and liquid tumors and can be robustly activated following treatment with inhibitors of DNA methyltransferase (DNMT) and histone deacetylase (HDAC). Here, we have analyzed MT-I chromatin structure in active, unmethylated (Hepa cells) and in repressed, methylated state (lymphosarcoma cells). Restriction enzyme accessibility assay showed that the MT-I promoter has an open conformation in unmethylated state as opposed to refractory chromatin structure in methylated state. Positioning of nucleosomal arrays on the methylated promoter further confirmed the closed chromatin structure of the methylated promoter. Chromatin immunoprecipitation (ChIP) assay demonstrated that the unmethylated promoter is associated with K9-acetyl, K4-methyl, and S10-phospho histone H3 whereas the methylated promoter is predominantly associated with K9-methyl H3. HP1alpha that recognizes K9-methyl H3 inhibited methylated MT-1 promoter activity whereas closely related HP1gamma repressed the promoter irrespective of its methylation status. Ubiquitously expressed DNA

methyltransferase 1 (DNMT1) suppressed MT-I promoter activity irrespective of its methylation status that does not require its catalytic activity. The DNMT1-mediated repression of MT-I promoter was relieved by trichostatin A, an HDAC inhibitor. Among the methyl CpG binding proteins, MBD2 and MBD4 specifically associated with the methylated promoter and inhibited its activity. In contrast, MBD1 and MeCP2 interacted with both promoters and suppressed the promoter activity irrespective of its methylation status. These results demonstrate that the methylated and unmethylated MT-I promoter are differentially regulated by DNA methyltransferase and methyl-CpG binding proteins, and DNMT1 could suppress MT promoter by a transcriptional mechanism independent of its enzymatic function. These studies suggest that the components of epigenetic machinery differentially regulate methylated and unmethylated MT-I gene expression.

Marie, V., et al. (2006). "Metallothionein gene expression and protein levels in triploid and diploid oysters Crassostrea gigas after exposure to cadmium and zinc." Environ Toxicol Chem 25(2): 412-418.

Quantitative real-time polymerase chain reaction (PCR) was used to compare for the first time the differential expression of metallothionein (MT) isoform genes, together with biosynthesis of the total MT proteins, in the gills of triploid and diploid juvenile Pacific oyster Crassostrea gigas in response to cadmium (Cd) and zinc (Zn) exposure. Oysters were exposed to Cd (0.133 microM), Zn (15.3 microM), and Cd+Zn for 14 d. Results showed similar response capacities to metal exposures in the two populations. No significant difference was revealed in terms of MT gene expression, MT protein synthesis, and Cd accumulation. However, triploid oysters bioaccumulated Zn 30% less efficiently than diploid oysters. Among the three MT isoform genes, CgMT2 appeared to be more expressed than CgMT1, whereas CgMT3 appeared to be anecdotal (10(6) times lower than CgMT2). CgMT2 and CgMT1 gene expression levels were increased sevenfold in the presence of Cd, whereas Zn appeared to have no effect. A twofold increase in MT protein levels occurred in response to Cd exposure. Discrepancies between mRNA and protein levels suggest that in C. gigas MT are regulated at the transcriptional level, as well as at the translational level.

Maroni, G., et al. (1986). "Molecular and cytogenetic characterization of a metallothionein gene of Drosophila." Genetics 112(3): 493-504.

A chromosomal DNA segment containing the metallothionein gene was isolated from a genomic library of Drosophila melanogaster using a previously characterized cDNA of this species as a probe. A segment of 1543 base pair (bp) was sequenced and found to include the cDNA sequence interrupted by one small intron. Several lines of evidence indicate that there is a single copy of the metallothionein gene (Mtn) in Drosophila; any other related genes, if they occur, must be sufficiently different that they are not detectable by our probe, even under hybridization conditions of reduced stringency. According to in situ hybridization and deletion mapping, Mtn is located in the right arm of the third chromosome in region 85E10-15. Within 300 bases upstream of the apparent site of transcription initiation, there are several short intervals very similar to the 12-bp segments considered to be responsible for metal regulation in mammalian systems.

Maroni, G., et al. (1987). "The metallothionein gene of Drosophila." Experientia Suppl 52: 385-392.

A chromosomal DNA segment containing the metallothionein gene was isolated from a genomic library of Drosophila melanogaster. A segment of 1543 bp was sequenced and found to include the structural sequence interrupted by one small intron. Within 300 bases upstream of the apparent site of transcription initiation, there are several short intervals very similar to the 12-base-pair segments considered to be responsible for metal regulation in mammalian systems. Several lines of evidence indicate that there is a single copy of the metallothionein gene (Mtn) in Drosophila. Mtn is located in the right arm of the third chromosome in region 85E10-15.

Maroni, G., et al. (1987). "Metallothionein gene duplications and metal tolerance in natural populations of Drosophila melanogaster." Genetics 117(4): 739-744.

A search for duplications of the Drosophila melanogaster metallothionein gene (Mtn) yielded numerous examples of this type of chromosomal rearrangement. These duplications are distributed widely--we found them in samples from four continents, and they are functional--larvae carrying Mtn duplications produce more Mtn RNA and tolerate increased cadmium and copper concentrations. Six different duplication types were characterized by restriction-enzyme analyses using probes from the Mtn region. The restriction maps show that in four cases the sequences, ranging in size between 2.2 and 6.0 kb, are arranged as direct, tandem repeats; in two other cases, this basic pattern is modified by the insertion of a putative transposable element into one of the repeated units. Duplications of the D. melanogaster metallothionein gene such as those that we found in natural populations may represent early stages in the evolution of a gene family.

Mauro, J. M. and M. Pazirandeh (2000). "Construction and expression of functional multi-domain polypeptides in Escherichia coli: expression of the Neurospora crassa metallothionein gene." Lett Appl Microbiol 30(2): 161-166.

A system for the construction of polymeric

peptides in Escherichia coli was utilized to prepare a library of plasmids coding for tandem repeats of the Neurospora crassa metallothionein gene. Selected oligomeric metallothionein clones were expressed and targeted to the periplasm as a fusion with the maltose-binding protein. Bacterial cells harbouring the expressed oligopeptides were characterized for their ability to bind 109Cd2+. The metal-binding ability was enhanced for all the oligomeric constructs tested and, in the best case, a 6.5-fold increased capacity for metal uptake was achieved with cells expressing a tandem 9-mer in comparison with cells expressing a monomer. Plateauing of the metal uptake ability occurred at between six and nine tandem repeats, possibly due to a combination of lowered translation levels, inefficient export and prematurely terminated translation products. The overall enhancement of the heavy metal removal capacity was approximately 65-fold relative to non-recombinant cells. The use of this strategy for the design and expression of de novo polypeptides containing multiple functional domains for use in bioremediation is discussed.

Mayo, K. E. and R. D. Palmiter (1982). "Glucocorticoid regulation of the mouse metallothionein I gene is selectively lost following amplification of the gene." J Biol Chem 257(6): 3061-3067.

The mouse metallothionein I (MT-I) gene is regulated by both heavy metals and glucocorticoid hormones. We selected cadmium-resistant variants of the mouse sarcoma cell line, S180, in which the metallothionein I gene has been amplified 10-fold and found that the amplified genes are regulated by cadmium in a manner identical with that observed for the original MT-I gene in unselected S180 cells. However, the amplified metallothionein I genes appear to be essentially nonresponsive to glucocorticoids even though at least 18 kilobases of DNA flanking the 5' side of the metallothionein I gene are amplified in these cells. The same result has been observed in nine clonal lines derived from the cadmium-resistant (CdR) population. The implications of this result both for models of steroid hormone action and for gene evolution are discussed.

Mayo, K. E., et al. (1982). "The mouse metallothionein-I gene is transcriptionally regulated by cadmium following transfection into human or mouse cells." Cell 29(1): 99-108.

Recombinant vectors containing the mouse metallothionein-I gene (MT-I) and the Escherichia coli xanthine-guanine phosphoribosyltransferase gene (gpt) were used to transfect human hgprt- HeLa cells. Transfected MT-I genes are transcriptionally regulated by cadmium but not by glucocorticoids. S1 mapping indicates that the transcripts from transfected MT-I genes begin at the correct transcription initiation site. We also transfected mouse tk- L cells with a vector containing the mouse MT- I gene and the herpes simplex virus-I thymidine kinase gene. MT-I gene transcription is regulated by cadmium but not by glucocorticoids in this homologous system as well. Finally, we fused the MT-I gene promoter/regulatory region to the thymidine kinase structural gene. Thymidine kinase activity is regulated by cadmium when this fusion gene is transfected into mouse tk- L cells. Deletion mapping experiments indicate that the DNA sequences necessary for regulation of the MT-I gene by cadmium lie within 148 bp of its transcription start site.

Mazzatti, D. J., et al. (2008). "Effects of interleukin-6 -174C/G and metallothionein 1A +647A/C single-nucleotide polymorphisms on zinc-regulated gene expression in ageing." Exp Gerontol 43(5): 423-432.

Decreased zinc ion availability in ageing is associated with altered immune response. One of the main regulators of zinc availability is metallothionein. Metallothionein induction is under the control of interleukin-6, a pro-inflammatory cytokine whose production is associated with poor ageing. The production of interleukin-6 is controlled, in part, by variability in the -174 nucleotide position. Under conditions of chronic inflammation, such as in ageing, zinc release by metallothionein is limited and may reduce zinc availability. Understanding the precise nature of the interactions between interleukin-6 and metallothioneins will aid in identifying individuals who are at risk of zinc deficiency. In the current study, we used gene arrays to investigate the effects of in vitro zinc supplementation on gene expression in elderly donors with described interleukin-6 and metallothionein 1a polymorphisms. Ingenuity Pathway Analysis identified several zinc-responsive genetic networks uniquely regulated only in elderly individuals with the pro-inflammatory interleukin-6 polymorphism. These include zinc-dependent decreased transcription of pro-inflammatory cytokines and alterations in metabolic regulatory pathways. The genomic effects of zinc increased in significance in the presence of the metallothionein 1a +647 C/A transition, suggesting that the interleukin-6 and metallothionein 1a genes act in a concerted manner to control zinc-regulated gene expression.

McKenna, I. M., et al. (1996). "Metallothionein gene expression in testicular interstitial cells and liver of rats treated with cadmium." Toxicology 107(2): 121-130.

The rodent testes are generally more susceptible to cadmium (Cd)-induced toxicity than the liver. Cd induces predominantly testicular interstitial cell (TIC) tumors. In order to clarify the molecular mechanism underlying tissue differences in Cd sensitivity, we compared Cd-induced metallothionein (MT) gene expression, MT protein accumulation, and

Cd retention in freshly isolated TICs and liver. Adult male Fischer rats received a s.c. injection of 4.0 micromol Cd/kg or vehicle and 24 h later tissues were sampled and TICs isolated. MT-I and MT-II mRNA levels were determined by slot-blot analysis followed by densitometry scanning, and MT was estimated by the Cd-heme method. Testicular lesions were not grossly or histologically observed in rats treated with 4 micromol Cd/kg. Both MT mRNA and MT (as determined by Cd-binding capacity) were constitutively present in TICs as well as the liver. TICs isolated from Cd-treated rats accumulated more Cd (4-fold), and had higher levels of MT-I (1.9-fold) and MT-II (1.4-fold) mRNAs over control, but contained less MT (30% decrease) than TICs isolated from control animals. Cd exposure substantially increased hepatic Cd content (6000-fold), MT (58-fold), and MT-I mRNA (5.3-fold), but did not increase MT-II mRNA. Thus, our findings indicate that, although low-dose Cd exposure results in increases of MT mRNA in TICs it does not enhance MT synthesis within these cells. The inability to induce the metal-detoxicating MT-protein, in response to Cd, might account for higher susceptibility of testes to Cd toxicity and carcinogenesis relative to liver.

McNeall, J., et al. (1989). "Hyperinducible gene expression from a metallothionein promoter containing additional metal-responsive elements." Gene 76(1): 81-88.

We describe the development of metallothionein-based vectors with low basal levels of expression that are hyperinducible upon treatment with heavy metals. Vectors were constructed by substituting a region in the hMTIIA promoter (bp -70 to -129) containing an element (BLE) involved in basal level expression with multiple metal responsive elements (MREs). In expression studies utilizing cat as a reporter gene, heavy metal inducibility was examined in both transiently transfected and permanently transformed Chinese hamster ovary (CHO) cells. Our results demonstrate that, within the same promoter structure, inducibility can be increased by altering the ratio of MREs to BLEs. Optimal induction of expression in permanently transformed CHO cells was achieved by exposure to heavy metals for 48 h prior to cell harvest, with an additional boost 12 h before harvest. These vectors have the potential to be used for production of proteins in cultured mammalian cells and in gene expression in transgenic animals.

Mehra, R. K., et al. (1990). "Selective and tandem amplification of a member of the metallothionein gene family in Candida glabrata." J Biol Chem 265(11): 6369-6375.

Metallothioneins constitute a multigene family in the yeast Candida glabrata. Two genes, designated metallothionein-I (MT-I) and one member of the metallothionein-II family (MT-II), were cloned and sequenced previously (Mehra, R. K., Garey, J. R., Butt, T. R., Gray, W. R., and Winge, D. R. (1989) J. Biol. Chem. 264, 19747-19753). Southern analysis of the genomic DNA samples from different wild-type isolates indicated that the MT-I gene was always present as a single copy but multiple (3-9) and tandemly arranged copies of one MT-II gene were present in different strains. Strains of C. glabrata highly resistant to copper salts were obtained by repeated culturing of wild-type isolates in medium containing increasing concentrations of copper sulfate. These strains showed further stable chromosomal amplification (greater than 30 copies) of the MT-II gene. The MT-I gene remained as a single copy. Amplified copies of the MT-II gene were always arranged tandemly. One of the copper-resistant strains acquired more copies of the MT-II gene by apparent duplication of the chromosome carrying this gene. The size of the amplification unit was 1.25 kilobases. The principal MT-I and -II genes of C. glabrata were shown to map to different chromosomes by electrophoretic karyotypic analysis. The length of chromosome carrying MT-II gene increased appreciably in strains exhibiting the highest amplification of this gene. Northern analysis showed increased basal levels of MT-II mRNA in strains having highly amplified MT-II locus.

Mehra, R. K., et al. (1992). "Cloning system for Candida glabrata using elements from the metallothionein-IIa-encoding gene that confer autonomous replication." Gene 113(1): 119-124.

The yeast Candida glabrata harbors two distinct gene families that encode metallothioneins (MTs). One of these loci, the MT-IIa locus, exhibits selective and tandem amplification in many wild type strains of C. glabrata. The present paper demonstrates that the amplified MT-IIa gene contains autonomously replicating sequences (ARS). These ARS elements have been used to construct vectors capable of replicating in C. glabrata. The ARS element(s) in the MT-IIa gene were localized to a 457-bp segment downstream from the MT-IIa coding sequence. Although plasmids containing this fragment transform C. glabrata with high frequency, the stability of the transformants and the copy number of the plasmid improve when the entire 1.25-kb MT-IIa gene is used. Transformation of C. glabrata with plasmids carrying the 2 microns circle ARS of Saccharomyces cerevisiae led to the formation of micro-colonies, indicating that the ARS elements of 2 microns plasmids replicate only to a limited extent in C. glabrata. Conversely, a C. glabrata plasmid carrying three copies of the MT-IIa gene was able to transform S. cerevisiae.

Mekawy, A. M. M., et al. (2020). "Constitutive overexpression of rice metallothionein-like gene

OsMT-3a enhances growth and tolerance of Arabidopsis plants to a combination of various abiotic stresses." J Plant Res 133(3): 429-440.

Metallothioneins (MT) are primarily involved in metal chelation. Recent studies have shown that MT proteins are also involved in the responses of plants to various environmental stresses. The rice metallothionein-like gene OsMT-3a is upregulated by salinity and various abiotic stressors. A DNA construct containing the complete OsMT-3a coding sequence cloned downstream to the CaMV35S promoter was transformed into Arabidopsis and homozygous single-copy transgenic lines were produced. Compared to wild-type plants, transgenic plants showed substantially increased salinity tolerance (NaCl), drought tolerance (PEG), and heavy metal tolerance (CdCl2) as individual stresses, as well as different combinations of these stresses. Relevantly, under unstressed control conditions, vegetative growth of transgenic plants was also improved. The shoot Na(+) concentration and hydrogen peroxide in transgenic plants were lower than those in wild-type plants. OsMT-3a-overexpressing Arabidopsis lines accumulated higher levels of Cd(2+) in both shoots and roots following CdCl2 treatment. In the transgenic MT-3a lines, increased activity of two major antioxidant enzymes, catalase and ascorbate peroxidase, was observed. Thus, rice OsMT-3a is a valuable target gene for plant genetic improvement against multiple abiotic stresses.

Mengheri, E., et al. (1993). "Metallothionein gene is expressed in developing rat intestine and is induced by zinc but not by corticosteroids." J Nutr 123(5): 817-822.

The expression of metallothionein (MT) mRNA during perinatal development of rat intestine and its induction by zinc and corticosteroids were studied. Pregnant rats from d 17 to 22 of gestation and rats at 2, 4, 13 and 21 d of postnatal life were injected with saline solution (control) or with zinc (10 mg/kg body wt) or corticosteroids (1 mg/kg body wt). After 6 h, tissues were removed for analysis. Northern hybridization of polyA + RNA to 32P-MT-cDNA revealed that MT was expressed already at d 17 of fetal life, increased afterwards (reaching the maximal expression around birth) and decreased soon after until weaning. Metallothionein mRNA was markedly induced by zinc at d 18 of fetal life to a level that remained constant throughout postnatal life. Corticosteroids were ineffective in inducing MT gene expression during prenatal and postnatal development. In 21-d-old adrenalectomized rats the level of MT mRNA was similar to that of control rats of the same age and was not changed by hormone treatment. The results indicate that MT gene expression can be induced by zinc during fetal life and that its expression without exogenous inducers cannot be ascribed to circulating corticosteroids.

Mercer, J. F., et al. (1992). "Hepatic metallothionein gene expression in toxic milk mice." J Nutr 122(6): 1254-1259.

The toxic milk mutation (tx) in mice is an autosomal recessive condition that causes a marked hepatic accumulation of copper in adults and severe copper deficiency in the pups of tx/tx dams. We determined the concentration of metallothionein-I (MT-I) mRNA in mutant and normal animals at various stages of development and following administration of copper and zinc. In two tx/tx males the average MT-I mRNA was 329 molecules/pg RNA compared with 38 molecules/pg in normal animals. In fetal and neonatal animals the concentration of MT-I mRNA was generally the same in normal and mutant mice and was independent of copper status. Copper or zinc administration to 7-d-old pups caused a marked induction of MT-I mRNA. There was an increased response to copper administration in one mutant group, but no clear pattern of hyper-induction of the MT gene in tx/tx animals was demonstrated. The elevation of MT-I mRNA in adult toxic milk mice is likely to be a secondary consequence of copper accumulation and not a primary effect of the mutation, because high MT-I mRNA levels would have been observed in the mutant neonates and fetuses. However, the possibility that the tx mutation causes overexpression of MT in post-weaning animals cannot be excluded by these data. The results also show that copper deficiency has no effect on the fetal or neonatal expression of the MT genes.

Merlos Rodrigo, M. A., et al. (2018). "Comparative gene expression profiling of human metallothionein-3 up-regulation in neuroblastoma cells and its impact on susceptibility to cisplatin." Oncotarget 9(4): 4427-4439.

Human metallothionein-3 (hMT-3), also known as growth inhibitory factor, is predominantly expressed in the central nervous system. hMT-3 is presumed to participate in the processes of heavy metal detoxification, regulation of metabolism and protection against oxidative damage of free radicals in the central nervous system; thus, it could play important neuromodulatory and neuroprotective roles. However, the primary functions of hMT-3 and the mechanism underlying its multiple functions in neuroblastoma have not been elucidated so far. First, we confirmed relatively high expression of hMT-3 encoding mRNA in biopsies (n = 23) from high-risk neuroblastoma subjects. Therefore, we focused on investigation of the impact of hMT-3 up-regulation in N-Myc amplifying neuroblastoma cells. The differentially up-regulated genes involved in biological pathways related to cellular senescence and cell cycle were identified using electrochemical microarray with consequent bioinformatic processing. Further, as experimental verification of microarray data, the cytotoxicity of the cisplatin (CDDP) was examined in hMT-3 and mock

cells by MTT and clonogenic assays. Overall, our data strongly suggest that up-regulation of hMT-3 positively correlates with the genes involved in oncogene-induced senescence (CDKN2B and ANAPC5) or apoptosis (CASP4). Moreover, we identified a significant increase in chemoresistance to cisplatin (CDDP) due to hMT-3 up-regulation (24IC50: 7.5 vs. 19.8 mug/ml), indicating its multipurpose biological significance.

Messaoudi, I., et al. (2010). "Evaluation of involvement of testicular metallothionein gene expression in the protective effect of zinc against cadmium-induced testicular pathophysiology in rat." Reprod Toxicol 29(3): 339-345.

To investigate the effects of exposure to Cd and Zn on testicular MT-1 and MT-2 gene expression and evaluate their involvement in Zn protection against Cd-induced testicular pathophysiology, male rats received either tap water, Cd or Cd+Zn in their drinking water for 35 days. Cd induced histopathological changes in testicular tissues were accompanied by decreased plasma testosterone level, plasma and testicular Zn concentrations, oxidative stress, and by increased MT-1 and MT-2 gene expression. Co-treatment with Cd and Zn reversed the Cd-induced decrease testosterone level and SOD activity, decreased testicular Cd accumulation and partially restored Cd-induced histological changes, lipid peroxidation, and Zn depletion. The increase of testicular MT-1 and MT-2 gene expression under Cd influence was significantly reduced in Cd+Zn group. These data suggest that Zn enhances the protection against Cd-induced testicular pathophysiology through non-MT gene expression mechanisms but essentially by preventing Cd accumulation, Zn deprivation and by ameliorating the testicular antioxidant status.

Michael, G. J., et al. (2011). "Up-regulation of metallothionein gene expression in parkinsonian astrocytes." Neurogenetics 12(4): 295-305.

The role of glial cells in Parkinson's disease (PD) is unclear. We have previously reported a striking up-regulation of DnaJB6 heat shock protein in PD substantia nigra astrocytes. Whole genome transcriptome analysis also indicated increased expression of metallothionein genes in substantia nigra and cortex of sporadic PD cases. Metallothioneins are metal-binding proteins in the CNS that are released by astrocytes and associated with neuroprotection. Metallothionein expression was investigated in 18 PD cases and 15 non-PD controls using quantitative real-time polymerase chain reaction (qRT-PCR), in situ hybridisation (ISH) and immunocytochemistry (ICC). We observed a strong increase in the expression of metallothioneins MT1E, MT1F, MT1G, MT1H, MT1M, MT1X and MT2A in both PD nigra and frontal cortex. Expression of LRP2 (megalin), the neuronal metallothionein receptor was also significantly increased. qRT-PCR confirmed metallothionein up-regulation. Astrocytes were found to be the main source of metallothioneins 1 and 2 based on ISH results, and this finding was confirmed by ICC. Our findings demonstrate metallothionein expression by reactive astrocytes in PD nigra and support a neuroprotective role for these cells. The traditional view that nigral astrocytes are non-reactive in PD is clearly incorrect. However, it is possible that astrocytes are themselves affected by the disease process which may explain their comparatively modest and previously overlooked response.

Mikowska, M., et al. (2018). "Variation of Metallothionein I and II Gene Expression in the Bank Vole (Clethrionomys glareolus) Under Environmental Zinc and Cadmium Exposure." Arch Environ Contam Toxicol 75(1): 66-74.

The main idea of the study was to assess how environmental metal pollution activates defence responses at transcription levels in the tissues of bank voles (Clethrionomys glareolus). For this purpose, the metallothioneine (MT) genes expression (a well known biomarker of exposure and response to various metals) was measured. The real-time PCR method was used for relative quantification of metallothionein I and metallothionein II expressions in the livers, kidneys and testes of bank voles from six populations exposed to different contaminants, mainly zinc, cadmium and iron. The assessment of Zn, Cu and Fe concentrations in the tissues allowed to study the MTs gene expression responses to these metals. ANOVA analysis showed differences between populations in terms of metal concentration in tissues, livers and kidneys. Student T test showed significant differences in metal concentration between unpolluted and polluted sites only for the liver tissue: significantly lower Zn levels and significantly higher Fe levels in the unpolluted sites. Kruskal-Wallis test performed on C T data shows differences in the gene expressions between populations for both MT genes for liver and testes. In the liver metallothionein I gene expression was upregulated in populations considered as more polluted (up to 7.5 higher expression in Miasteczko Slaskie comparing to Mikolajki). Expression of metallothionein II revealed a similar pattern. In kidneys, differences in expression of both MT genes were not that evident. In testes, MT upregulation in polluted sites was noted for metallothionein II. For metallothionein however, we found downregulation in populations from more contaminated sites. The expressions of both MTs were positively influenced by cadmium in kidney (concentration data from the previous study) and zinc and copper in liver, while cadmium had effects only on the liver MT II gene expression. Positive relationship was obtained for lead

and metallothionein II expression in the liver.
Miller, K. F., et al. (1989). "Expression of human or bovine growth hormone gene with a mouse metallothionein-1 promoter in transgenic swine alters the secretion of porcine growth hormone and insulin-like growth factor-I." J Endocrinol 120(3): 481-488.

Endocrine profiles were examined in swine that had integrated and expressed a fusion gene consisting of mouse metallothionein-1 (MT) promoter fused to either a human (h) or bovine (b) GH structural gene. Eleven of 18 pigs that had integrated MT-hGH and eight of nine pigs that had integrated MT-bGH expressed the genes. The level of expression varied widely among pigs (14-4551 micrograms/l for MT-hGH and 23-1578 micrograms/l for MT-bGH). The level of expression varied over time within each pig with no general pattern. Concentrations of porcine GH (pGH) were lower in MT-hGH pigs that expressed the gene than in non-expressors or in littermate controls. Insulin-like growth factor-I (IGF-I) concentrations increased with age in all pigs and were raised threefold in pigs expressing either the MT-hGH or MT-bGH genes. Measurement of the foreign GH in samples taken at 15-min intervals failed to reveal any short-term fluctuations in concentration. Administration of hGH releasing factor (GRF) to pigs expressing MT-bGH resulted in attenuated release of pGH compared with that of contemporary controls. Concentrations of bGH did not change after GRF injection. Human and bovine GH expressed in transgenic pigs appear to be biologically active in that they induce IGF-I and suppress endogenous pGH secretion. The failure to find short-term fluctuations and the lack of response to GRF injections are consistent with a non-pituitary and non-GRF regulatable site of production.

Minichiello, L., et al. (1994). "Interactions of nuclear proteins from uninduced, induced and superinduced HeLa cells with metal regulatory elements MRE3 and 4 of the human metallothionein IIa-encoding gene." Gene 143(2): 289-294.

Transcriptional activation of metallothionein (MT)-encoding genes(MT) is regulated during heavy metal induction by short non-identical repeats, termed 'metal regulatory elements' (MRE), present in multiple imperfect copies in MT promoter regions of eukaryotes. Using mobility shift assays, we have studied the interaction between the human MRE 3 and 4 regions (hMRE3/4) of the MTIIa promoter and nuclear proteins from uninduced and Cd(2+)-induced HeLa cells, and from Cd(2+)-superinduced H454 cells, a HeLa-derived Cd(2+)-resistant cell isolate which overexpresses hMTIIa after exposure to metal. A specific complex with a similar electrophoretic mobility was formed in all three extracts. Dialysis of the extracts using EDTA inhibited the formation of the complexes, which could be reconstituted only after the addition of Zn2+. UV cross-linking analyses of the specific complexes formed by the three nuclear extracts interacting with the hMRE3/4 region revealed that in all of them polypeptides were present having similar electrophoretic mobilities and different molecular masses. Mobility shift assays showed no major differences in the binding of nuclear proteins from induced or uninduced cells. Proposed models of activation of metal-induced MT transcription are discussed.

Miura, N. (2009). "Individual susceptibility to cadmium toxicity and metallothionein gene polymorphisms: with references to current status of occupational cadmium exposure." Ind Health 47(5): 487-494.

The incidence of serious poisoning caused by occupational cadmium exposure has declined over the past four decades due to improvements in the work environment. However, long-term low-level exposure to cadmium needs to be addressed. For workers in industries that handle cadmium, it is necessary to consider the daily cadmium intake from contaminated foods such as cereals and rice in addition to the occupational exposure, since workers might be exposed to higher levels of cadmium from a combination of these sources. Cadmium accumulates in the renal cortex by the long-term exposure along with increased concentrations of metallothionein, an important protein for protection from cadmium toxicity. However, some individuals have lower metallothionein levels despite increased cadmium accumulation in the kidneys. This article describes the strategy method for analyzing individual susceptibility to cadmium toxicity and genetic polymorphisms of metallothionein, with reference to the current status of occupational cadmium exposure.

Miura, N. and S. Koizumi (2005). "Gene expression profiles in the liver and kidney of metallothionein-null mice." Biochem Biophys Res Commun 332(4): 949-955.

It has been reported that the expression of certain genes was altered in rodent cells lacking metallothioneins (MTs). To further explore the effects of MT deficiency, we screened genes differentially expressed in the liver and kidney of MT-null mice by cDNA microarray analysis. In the liver, 29 of 8737 genes analyzed were altered in their expression levels: 19 and 10 genes were up-regulated and down-regulated, respectively. Particularly, 14 of the 29 genes were related to energy metabolism, and some of these suggested that loss of MTs might lead to obesity and irregular ATP synthesis. In the kidney, 41 differentially expressed genes were observed: 27 and 14 genes were up-regulated and down-regulated, respectively. Eleven of the 41 genes were also related to energy metabolism. Microarray results were confirmed by Northern blot

analysis for five of the energy metabolism-related genes.

Miura, N. and A. Naganuma (2000). "Metallothionein mediates gene expression of 3.1 mRNA (PTZ17) related to epileptic seizure." FEBS Lett 479(3): 146-148.

Genes differentially expressed in association with disruption of the metallothionein gene were screened using two hepatic stellate cell lines isolated and established from the livers of normal 129/Sv (IMS/N cells) and transgenic mice deficient in the genes for metallothionein-I and -II (IMS/MT (-) cells). We found one cDNA (tentatively named NM31) that was expressed only in IMS/IN cells. Transfecting IMS/MT (-) cells with the genes for both metallothionein-I and -II resulted in NM31 expression. These results suggest that metallothionein is essential for NM31 gene expression. The nucleotide sequence of NM31 (294 bp) was identical to the 3' region of 3.1 mRNA (PTZ 17), which is abundant in the embryonic mouse brain and is related to chemically induced seizures. The present study indicates that metallothionein mediates the expression of specific genes. This is a novel explanation for some of the functions of metallothionein.

Miyashita, N. T., et al. (2005). "DNA variation in the metallothionein genes in wild rice Oryza rufipogon: relationship between DNA sequence polymorphism, codon bias and gene expression." Genes Genet Syst 80(3): 173-183.

This study examines the relationship between DNA sequence variation and level of gene expression in four metallothionein genes from wild rice Oryza rufipogon. The nucleotide diversity was 0.0028 to 0.0117 over the entire coding and non-coding region, and it was negatively correlated with gene expression for three type 2 metallothionein genes. In contrast, codon bias and percent of preferred codons correlated positively with gene expression. These results indicate that the intensity of natural selection depends on the level of gene expression, which in turn shapes the level of nucleotide polymorphism. In addition, significant linkage disequilibria were frequent between the metallothionein genes, although significance was not confirmed after multiple test correction. This result suggests that metallothionein genes expressed at different levels are epistatic with respect to fitness, and that gene expression is an important factor determining level of DNA polymorphism.

Moffatt, P., et al. (1995). "Induction of metallothionein gene expression by epidermal growth factor and its inhibition by transforming growth factor-beta and dexamethasone in rat hepatocytes." Hepatology 21(4): 1038-1044.

Metallothionein (MT) is a small cysteine-rich protein thought to be mainly involved in metal regulation and detoxification. The implication of MT in cell growth and differentiation has also been suggested. This latter hypothesis was further investigated in adult rat hepatocytes induced to proliferate by epidermal growth factor (EGF). Exposure of hepatocytes to EGF resulted in significant increases (approximately twofold) in MT protein and MT-1 messenger RNA (mRNA) levels, which were maximal after 48 hours. As revealed by nuclear run-on analysis, these changes were the result of transcriptional activation. Increases of MT occurred concomitantly with stimulation of DNA synthesis (48 hours). Addition of ZnSO4 or dexamethasone (Dex) was also effective at inducing MT protein (approximately 3.6 to 3.3 times) and mRNA. Combined addition of Zn and EGF produced an additive increase in MT protein and MT-1 mRNA levels. When both Dex and EGF were present together, the EGF-induced MT protein and mRNA expression was lost, whereas it had only minor inhibitory effects on DNA synthesis. Transforming growth factor beta (TGF-beta), a known antagonist of EGF on hepatocytes, blocked the EGF-induced MT accumulation and stimulation of DNA synthesis. In addition, under the same conditions, the EGF-induced c-fos mRNA accumulation was blocked by Dex whereas TGF-beta had no effect. These results show that growth factors believed to play a role in liver regeneration can also modulate MT gene expression in vitro. This modulation does not strictly parallel that of DNA synthesis. The possibility that c-fos stimulation may play a role in MT induction by EGF cannot be ruled out.

Moffatt, P. and C. Seguin (1998). "Expression of the gene encoding metallothionein-3 in organs of the reproductive system." DNA Cell Biol 17(6): 501-510.

Metallothionein-3 (MT-3) is a new MT gene-family member that inhibits survival of rat neurons cultured in presence of brain extracts. Contrary to other MT genes, which are expressed in most tissues and which are highly inducible by metals, MT-3 expression was reported to be mainly in the brain, and it failed to respond to metals in vivo. We show here that MT-3 mRNA is present in several organs other than the brain, as assayed by Northern analyses. In the rat, MT-3 mRNA was detected in the testis, prostate, epididymis, tongue, ovary, uterus, stomach, heart, and seminal vesicles. The MT-3 mRNA levels in the testis, epididymis, prostate, and tongue were 22% of those in brain, while in ovary, uterus, and stomach, they were 4% of the brain level, and they were lower still in the other organs. The MT-3 gene was not inducible by CdCl2 or lipopolysaccharide in rat testis and prostate. In the mouse and the human, relative MT-3 mRNA levels were lower than those found in the rat when compared with those present in brain. Testicular MT-3 transcript levels remained quite constant during rat postnatal

development in animals aged from 6 to 43 days. In situ hybridization analyses on human testis sections showed that MT-3 mRNA was present at different levels in both the Leydig cells and the seminiferous tubules. In orchiectomized rats, prostatic MT-3 mRNA was decreased by 75%, and injections of dihydrotestosterone restored MT-3 mRNA levels to control values. Overall, these results show that MT-3 tissue-specific gene expression is broader than previously reported and provide new experimental systems to study the function and mechanism of action of the MT-3 protein.

Moilanen, L. H., et al. (1999). "Regulation of metallothionein gene transcription. Identification of upstream regulatory elements and transcription factors responsible for cell-specific expression of the metallothionein genes from Caenorhabditis elegans." J Biol Chem 274(42): 29655-29665.

Metallothioneins are small, cysteine-rich proteins that function in metal detoxification and homeostasis. Metallothionein transcription is controlled by cell-specific factors, as well as developmentally modulated and metal-responsive pathways. By using the nematode Caenorhabditis elegans as a model system, the mechanism that controls cell-specific metallothionein transcription in vivo was investigated. The inducible expression of the C. elegans metallothionein genes, mtl-1 and mtl-2, occurs exclusively in intestinal cells. Sequence comparisons of these genes with other C. elegans intestinal cell-specific genes identified multiple repeats of GATA transcription factor-binding sites (i.e. GATA elements). In vivo deletion and site-directed mutation analyses confirm that one GATA element in mtl-1 and two in mtl-2 are required for transcription. Electrophoretic mobility shift assays show that the C. elegans GATA transcription factor ELT-2 specifically binds to these elements. Ectopic expression of ELT-2 in non-intestinal cells of C. elegans activates mtl-2 transcription in these cells. Likewise, mtl-2 is not expressed in nematodes in which elt-2 has been disrupted. These results indicate that cell-specific transcription of the C. elegans metallothionein genes is regulated by the binding of ELT-2 to GATA elements in these promoters. Furthermore, a model is proposed where ELT-2 constitutively activates metallothionein expression; however, a second metal-responsive factor prevents transcription in the absence of metals.

Moleirinho, A., et al. (2011). "Gains, losses and changes of function after gene duplication: study of the metallothionein family." PLoS One 6(4): e18487.

Metallothioneins (MT) are small proteins involved in heavy metal detoxification and protection against oxidative stress and cancer. The mammalian MT family originated through a series of duplication events which generated four major genes (MT1 to MT4). MT1 and MT2 encode for ubiquitous proteins, while MT3 and MT4 evolved to accomplish specific roles in brain and epithelium, respectively. Herein, phylogenetic, transcriptional and polymorphic analyses are carried out to expose gains, losses and diversification of functions that characterize the evolutionary history of the MT family. The phylogenetic analyses show that all four major genes originated through a single duplication event prior to the radiation of mammals. Further expansion of the MT1 gene has occurred in the primate lineage reaching in humans a total of 13 paralogs, five of which are pseudogenes. In humans, the reading frame of all five MT1 pseudogenes is reconstructed by sequence homology with a functional duplicate revealing that loss of invariant cysteines is the most frequent event accounting for pseudogeneisation. Expression analyses based on EST counts and RT-PCR experiments show that, as for MT1 and MT2, human MT3 is also ubiquitously expressed while MT4 transcripts are present in brain, testes, esophagus and mainly in thymus. Polymorphic variation reveals two deleterious mutations (Cys30Tyr and Arg31Trp) in MT4 with frequencies reaching about 30% in African and Asian populations suggesting the gene is inactive in some individuals and physiological compensation for its loss must arise from a functional equivalent. Altogether our findings provide novel data on the evolution and diversification of MT gene duplicates, a valuable resource for understanding the vast set of biological processes in which these proteins are involved.

Morahan, J. M., et al. (2005). "Screening the metallothionein III gene in sporadic amyotrophic lateral sclerosis." Amyotroph Lateral Scler Other Motor Neuron Disord 6(2): 115-117.

Metallothioneins are proteins involved in antioxidant defence, essential metal homoeostasis and heavy metal detoxification, all mechanisms implicated in sporadic amyotrophic lateral sclerosis (SALS). We therefore looked for changes in the gene for nervous system-specific metallothionein III (MT3) that might explain susceptibility to SALS. DNA was extracted from 87 sporadic ALS and 174 matched controls. The gene for MT3 was sequenced in 20 SALS and 5 control subjects to identify single nucleotide polymorphisms (SNPs). These SNPs were then screened in all subjects. Eight novel SNPs were found in the 5' untranslated region and intron 2 of MT3. No differences were found in the frequency distribution of alleles or haplotypes for these SNPs between the SALS and control groups. The genotype distribution of one SNP (A1422C) was significantly different between ALS and control groups ($p<0.02$) but this is not likely to be biologically relevant. We conclude that changes in the MT3 gene are unlikely to be responsible for susceptibility to SALS.

Morby, A. P., et al. (1993). "SmtB is a metal-dependent repressor of the cyanobacterial metallothionein gene smtA: identification of a Zn inhibited DNA-protein complex." Nucleic Acids Res 21(4): 921-925.

The smt locus of Synechococcus PCC 7942 contains a metal-regulated gene (smtA), which encodes a class II metallothionein, and a divergently transcribed gene, smtB, which encodes a repressor of smtA transcription. Regions containing cis-acting elements required for efficient induction, and required for smtB-dependent repression, of the smtA operator-promoter were identified. Specific interactions between proteins extracted from Synechococcus PCC 7942 and defined regions surrounding the smtA operator-promoter were detected by electrophoretic mobility shift assays. Three metallothionein operator-promoter associated complexes were identified, one of which (MAC1) showed Zn-dependent dissociation and involved a region of DNA immediately upstream of smtA. Treatment with Zn-chelators facilitated re-association of MAC1 in vitro. MAC1 was not observed in extracts from smt deficient mutants but was restored in extracts from mutants complemented with a plasmid borne smtB. SmtB is thus required for the formation of a Zn-responsive complex with the smt operator-promoter and based upon the predicted structure of SmtB we propose direct SmtB-DNA interaction exerting metal-ion inducible negative control.

Moriguchi, T., et al. (1998). "Characterization of gene repertoires at mature stage of citrus fruits through random sequencing and analysis of redundant metallothionein-like genes expressed during fruit development." Gene 211(2): 221-227.

We carried out a random sequencing of cDNA library derived from mature citrus fruit (Citrus unshiu Marc.) for identifying the gene repertoires expressed at the mature stage. Among 297 clones analyzed, 195 cDNA clones (65.7%) were putatively identified to previously characterized genes with optimized (OPT) scores of >/=200 through a homology search to DNA database, whereas 102 clones (34.3%) resulted in low OPT scores (<200) and did not show any significant sequence identity with previously published genes. Among them, clones homologous to metallothionein (MT)-like genes appeared 62 times, being mostly redundant, and accounting for about 20.9% of the total 297 clones. To gain a better understanding of the MT-like genes, two types of cDNA clones were isolated. One clone (CitMT36) resembled the type 2 MT gene containing Cys-X-Cys motifs in both N- and C-terminal, but the consensus sequence in the N-terminal domain, Cys-Cys and Cys-X-X-Cys was modified in CitMT36 to X-Cys and Cys-X-X-X, respectively. We suggest that these form a 'novel type 2' group of MT-like clones. The other clone (CitMT45) showed homology to type 3 MT-like genes, which have been found in mostly fruit tissues so far. By Southern blot analysis, both clones showed one or two bands, suggesting that both CitMT36 and CitMT45 are present in single or a few copies in the citrus genome. Transcripts of CitMT36 were evenly detected in all tissues examined, whereas those of CitMT45 were detected primarily in fruit during the developmental phase. Neither of the MT-like genes was induced in leaves by Zn and Cu. Collectively, MT-like genes from citrus would be regulated differentially depending on the fruit developmental stage and organs, indicating a change in their expression under the different physiological and molecular environment of fruit cells.

Mudalkar, S., et al. (2014). "Molecular cloning and characterisation of metallothionein type 2a gene from Jatropha curcas L., a promising biofuel plant." Mol Biol Rep 41(1): 113-124.

In the present study, we have cloned a gene encoding JcMT2a protein from Jatropha curcas L., a promising biofuel tree species. Full length sequence of JcMT2a gene was isolated using RACE PCR. Heterologous expression of JcMT2a in Escherichia coli and its purification has shown distinct bands corresponding to the GST and GST-fused JcMT2a protein. Significant tolerance was observed in E. coli cells expressing recombinant GST-JcMT2a for zinc, copper and cadmium metals compared to cells expressing GST alone. JcMT2a also restored Cu and Cd tolerance in the metal sensitive yeast mutants. Quantitative real time PCR showed a significant increase in JcMT2a transcripts with Cu and Cd in the leaf compared to root tissue. Our Scanning electron microscopy and energy dispersive X-ray spectroscopy analysis clearly demonstrates that J. curcas L. could be a potential candidate for phytoremediation to clean heavy metals from the environment, in addition to its non-edible oil seed yields for biodiesel production.

Mullin, C. H., et al. (1988). "Metallothionein gene expression in mouse tissues by D-penicillamine." J Trace Elem Electrolytes Health Dis 2(1): 43-47.

The effect of D-penicillamine on metallothionein mRNA accumulation was examined in mouse tissues by Northern and dot blot analysis. This drug was given as a single intraperitoneal dose of 250 mg/kg body weight and the metallothionein mRNA content of the tissues was measured 1, 4, 8 and 24 hours later. A detectable increase of mRNA was observed after 1 hour and maximal accumulation was seen after 4 hours in the liver, kidneys, lungs, brain and spleen, whereas in the heart the maximum occurred after 8 hours. In the liver metallothionein mRNA was increased 14.5-fold over the control and in the kidneys it was increased by a factor of 9.2. A significant increase was also seen in the lungs, where it was 10 fold. To determine whether the increase is due to new transcription of the metallothionein gene, animals were

pretreated with actinomycin D (1.0 mg/kg body weight) before receiving D-penicillamine. Actinomycin D prevented some of the D-penicillamine-induced increase in metallothionein mRNA, indicating that the drug, to some extent, acts at the transcriptional level. Regulation of metallothionein gene expression may play an important role in the molecular mechanisms involved in the clinical action of D-penicillamine in rheumatoid arthritis.

Munger, K., et al. (1985). "Isolation and structural organization of the Neurospora crassa copper metallothionein gene." EMBO J 4(10): 2665-2668.

The Neurospora crassa copper metallothionein gene was cloned and its complete nucleotide sequence is reported. Enriched metallothionein mRNA was used as a template for cDNA synthesis, primed by a metallothionein-specific, synthetic undecanucleotide. The sequence of the cDNA obtained allowed the synthesis of a unique 21-mer which was used to screen a genomic DNA library of N. crassa. In agreement with the published amino acid sequence, the gene codes for a polypeptide 26 amino acid residues in length. The coding region is interrupted by a small intron (94 nucleotides). The gene structure is compared with those of mammalian metallothioneins. In both cases, the coding regions are split by introns, the intron-exon boundaries, however, are in different positions. The neurospora copper metallothionein gene is, to our knowledge, the smallest gene interrupted by an intron isolated so far.

Munger, K., et al. (1987). "Isolation and regulation of expression of the Neurospora crassa copper metallothionein gene." Experientia Suppl 52: 393-400.

The N. crassa CuMT gene has been cloned and its nucleotide sequence determined. To this end an MT specific undecanucleotide was synthesized and used for cDNA synthesis with enriched MT mRNA as a template. Sequence analysis of the cDNA obtained allowed the synthesis of a unique 21mer which was used as a hybridization probe to screen a genomic DNA library of N. crassa. Several positive clones were isolated and subjected to restriction and sequence analysis. In agreement with the published amino acid sequence, the gene codes for a polypeptide of 26 amino acid residues in length. The coding region is interrupted by a small intron. Compared to the structure of mammalian MT genes the intron-exon boundaries are located in different sequence positions. The induction of MT mRNA was studied by Northern analysis. Maximum levels of MT mRNA were detected about 1 hour after addition of copper ions to mycelium of N. crassa. The half-life time of the messenger was estimated as 2.5 hours. The CuMT amounts reach a maximum level at 3 hours after induction and thereafter remain constant.

Munger, K., et al. (1987). "The Neurospora crassa metallothionein gene. Regulation of expression and chromosomal location." J Biol Chem 262(15): 7363-7367.

The promoter region of the Neurospora crassa metallothionein gene contains no sequences which are similar to the mammalian or the yeast metal responsive elements (Munger, K., Germann, U. A., and Lerch, K. (1985) EMBO J. 4, 2665-2668). We therefore studied the regulation of expression of the N. crassa metallothionein gene in response to different metal ions (Cu2+, Cd2+, Zn2+, Co2+, and Ni2+) by Northern analysis. Only copper led to the induction of metallothionein mRNA. In N. crassa cultures inoculated and grown in copper-supplemented media, metallothionein mRNA appeared during the late logarithmic growth period (about 30 h after inoculation) and was detectable for a time period of more than 30 h. In response to copper shock, however, rapidly increasing amounts of metallothionein mRNA were detected within minutes after copper administration at any time in vegetatively growing mycelia of N. crassa. Maximum levels were detected about 1 h after addition of copper to the medium. The half-life time of the mRNA was estimated as 2.5 h. The amounts of copper metallothionein reach a maximum level at 3 h after induction and thereafter remain constant. The rapid induction by copper ions of metallothionein mRNA and metallothionein together with the remarkable stability of the native protein intracellularly suggest that this protein serves an important homeostatic role in the copper metabolism in this fungus. The structural gene of N. crassa metallothionein has been located on chromosome VI using restriction fragment-length polymorphisms as genetic markers.

Murphy, A. and L. Taiz (1995). "Comparison of metallothionein gene expression and nonprotein thiols in ten Arabidopsis ecotypes. Correlation with copper tolerance." Plant Physiol 109(3): 945-954.

Seedlings of 10 Arabidopsis ecotypes were compared with respect to copper tolerance, expression of two metallothionein genes (MT1 and MT2), and nonprotein thiol levels. MT1 was uniformly expressed in all treatments, and MT2 was copper inducible in all 10 ecotypes. MT1 and MT2 mRNA levels were compared with various growth parameters for the 10 ecotypes in the presence of 40 microM Cu2+. The best correlation (R = 0.99) was obtained between MT2 mRNA and the rate of root extension. MT2 mRNA levels also paralleled the recovery phase following inhibition by copper. Induction of MT2 mRNA was initiated at copper concentrations below the threshold for growth inhibition. In cross-induction experiments, Ag+, Cd2+, Zn2+, Ni2+, and heat shock all induced significant levels of MT2 gene expression, whereas Al3+ and salicylic acid did not. The correlation between copper tolerance and nonprotein thiol levels in

the 10 ecotypes was not statistically significant. However, 2 ecotypes, Ws and Enkheim, previously shown to exhibit an acclimation response, had the highest levels of nonprotein thiols. We conclude that MT2 gene expression may be the primary determinant of ecotypic differences in the copper tolerance of nonpretreated Arabidopsis seedlings.

Murphy, B. J., et al. (1999). "Activation of metallothionein gene expression by hypoxia involves metal response elements and metal transcription factor-1." Cancer Res 59(6): 1315-1322.

Metallothioneins (MTs) are a family of stress-induced proteins with diverse physiological functions, including protection against metal toxicity and oxidants. They may also contribute to the regulation of cellular proliferation, apoptosis, and malignant progression. We reported previously that the human (h)MT-IIA isoform is induced in carcinoma cells (A431, SiHa, and HT29) exposed to low oxygen, conditions commonly found in solid tumors. The present study demonstrates that the genes for hMT-IIA and mouse (m)MT-I are transcriptionally activated by hypoxia through metal response elements (MREs) in their proximal promoter regions. These elements bind metal transcription factor-1 (MTF-1). Deletion and mutational analyses of the hMT-IIA promoter indicated that the hMRE-a element is essential for basal promoter activity and for induction by hypoxia, but that other elements contribute to the full transcriptional response. Functional studies of the mMT-I promoter demonstrated that at least two other MREs (mMRE-d and mMRE-c) are responsive to hypoxia. Multiple copies of either hMRE-a or mMRE-d conferred hypoxia responsiveness to a minimal MT promoter. Mouse MT-I gene transcripts in fibroblasts with targeted deletions of both MTF-1 alleles (MTF-1(-/-); dko7 cells) were not induced by zinc and showed low responsiveness to hypoxia. A transiently transfected MT promoter was unresponsive to hypoxia or zinc in dko7 cells, but inductions were restored by cotransfecting a mouse MTF-1 expression vector. Electrophoretic mobility shift assays detected a specific protein-DNA complex containing MTF-1 in nuclear extracts from hypoxic cells. Together, these results demonstrate that hypoxia activates MT gene expression through MREs and that this activation involves MTF-1.

Murphy, M. F., et al. (1990). "Nucleotide sequence of the trout metallothionein A gene 5' regulatory region." Nucleic Acids Res 18(15): 4622.

Mustonen, M., et al. (2014). "Metallothionein gene expression differs in earthworm populations with different exposure history." Ecotoxicology 23(9): 1732-1743.

Metals are persistent pollutants in soils that can harm soil organisms and decrease species diversity. Animals can cope with metal contamination with the help of metallothioneins, small metal-binding proteins involved in homeostasis and detoxification of metals. We studied the expression of metallothionein with qPCR in a small, epigeic earthworm, Dendrobaena octaedra. We compared expression patterns and metal body content in earthworms collected from two sites with different metal contamination histories: Harjavalta, contaminated by a Cu-Ni smelter operational for over 50 years, and Jyvaskyla, an uncontaminated site. Earthworms from both sites were also experimentally exposed to different concentrations of Cu (control, 50, 100 or 200 mg/kg) or Zn (control, 75, 150 or 300 mg/kg) for 7, 14 or 28 days to determine if there is a time related dose-response in gene expression. Population comparison showed that metallothionein expression was higher in earthworms from the contaminated site. In the exposure experiment, exposure time affected expression, but only in the earthworms from the uncontaminated site, suggesting that there is a delay in the metallothionein response of earthworms in this population. In contrast, earthworms from the contaminated site showed higher and constant levels of metallothionein expression at all exposure concentrations and durations. The constant metallothionein expression in earthworms from the contaminated site suggests that inducibility of metallothionein response could be lost in earthworms with metal exposure history. Adaptation of D. octaedra to metal exposure could explain the differences between the populations and explain the persistence of this species in contaminated forest soils.

Nagasaki, H., et al. (2002). "Overexpression of vasopressin in the rat transgenic for the metallothionein-vasopressin fusion gene." J Endocrinol 173(1): 35-44.

Arginine vasopressin (AVP) is a major antidiuretic hormone, the overproduction of which causes diluting hyponatremia in humans and is called the syndrome of inappropriate antidiuresis (SIAD). To study physiological changes resulting from AVP overproduction and to develop an animal model of hyponatremia, the human AVP gene was expressed under the control of the metallothionein promoter in transgenic (Tg) rats. Analyses of AVP immunoreactivity (irAVP) in the tissues revealed that the transgene is expressed mainly in the central nervous system. Gel filtration showed that irAVP in the brain and plasma was properly processed AVP. AVP purified from the brains of both Tg and control rats also exerted equal bioactivity to generate cAMP in LLC-PK1 cells. The founder rats did not show any physical or anatomical abnormalities. Under basal conditions, Tg rats had high plasma AVP levels (Tg 13.8 +/- 2.5 pg/ml; control 2.7 +/- 1.2 pg/ml; n=6 in both groups; means +/- S.E.M.), decreased urine volume, and normal plasma [Na(+)]. Hypertonic saline injected i.p. did not

affect AVP secretion in Tg rats. In response to a zinc-supplemented liquid diet, plasma AVP decreased in control rats, but increased in Tg rats (Tg 32.7 +/- 2.7 pg/ml; control 1.0+/-0.1 pg/ml; n=6), resulting in hyponatremia (Tg 135.2 +/- 2.5 mEq/l; control 140.8 +/- 0.4 mEq/l; n=6). To our knowledge, this is the first transgenic animal to show diluting hyponatremia. This transgenic rat may therefore provide a useful model in which to investigate various physiological alterations resulting from the oversecretion of AVP which involve SIAD, stress response, behavior, and blood pressure.

Nakane, H., et al. (2015). "Impact of metallothionein gene polymorphisms on the risk of lung cancer in a Japanese population." Mol Carcinog 54 Suppl 1: E122-128.

Metallothioneins (MTs) are cysteine-rich proteins that act as antioxidants. A case-control study was conducted to assess the effects of gene polymorphisms in the MT region on the risk of lung cancer in Japanese subjects: 769 lung cancer cases and 939 non-cancer controls. Associations were evaluated using logistic regression models with adjustment for potential confounders (age, sex, and lifestyle factors including smoking, drinking, and green-yellow vegetable intake). We found five polymorphisms in the MT-1 gene region that showed statistically significant associations with lung cancer. Of these polymorphisms, rs7196890 showed the strongest association (odds ratio: 1.30, P = 0.004, 95% confidence interval: 1.09-1.55). The impact of the polymorphism decreased with the increase of smoking, and virtually no association with lung cancer was observed among heavy smokers whose pack-year values were 30 or more (odds ratio: 1.02, P = 0.93, 95% confidence interval: 0.67-1.55). These results suggest that polymorphisms in the MT gene are moderately associated with the risk of lung cancer and that the associations are modified by lifestyle factors.

Nakano, H., et al. (2006). "Human metallothionein gene expression is upregulated by beta-thujaplicin: possible involvement of protein kinase C and reactive oxygen species." Biol Pharm Bull 29(1): 55-59.

Recently, we discovered that beta-thujaplicin (BT) induces metallothionein (MT) expression in mouse keratinocytes, both in vivo and in vitro. However, the molecular mechanisms by which BT exerts its biological effects have not been elucidated. The purpose of this study is to explore the signal transduction pathway involved in the MT mRNA induction by BT. Using a HaCaT keratinocyte cell line, Northern blotting was performed for analyzing the human MT-IIA mRNA expression levels in combination with BT and a number of protein kinase (PK) inhibitors including H7, HA1004 and a PKC-specific inhibitor chelerythrin. CAT assays with the MT-IIA gene promorter-CAT construct were conducted for examining the transcriptional regulation by BT of MT. A free radical scavenger N-acetylcysteine (NAC) was used for analyzing a role of oxidative stress for the MT gene induction by BT. BT increased MT-IIA gene transcript levels and CAT activity in a dose-dependent fashion in HaCaT cells. The increase in MT-IIA mRNA levels and CAT activity were completely suppressed by H7 but not by HA1004. In addition, chelerythrin prevented BT-inducible MT-IIA promoter activation. Furthermore, NAC suppressed BT-inducible MT-IIA promoter activation. These results demonstrate that BT is a potent activator of the MT-IIA gene promoter and that PKC activation and reactive oxygen species are implicated in BT-inducible MT-IIA gene expression. BT may be a useful tool for dissecting the signal transduction pathway mediating MT-IIA promoter activation.

Nebes, V. L., et al. (1988). "Cyclic AMP induces metallothionein gene expression in rat hepatocytes but not in rat kidney." Biochem J 255(2): 741-743.

Cyclic AMP analogues induced expression of metallothionein-I (MT-I) mRNA in adult rat liver and in rat hepatocytes cultured in serum-free medium. This induction occurred via an increased rate of transcription of the MT-I gene. The effect of cyclic AMP analogues was tissue-specific since no induction of MT-I mRNA occurred in rat kidney. Induction of MT-I mRNA by a combination of cyclic AMP analogue and dexamethasone was additive in liver and cultured hepatocytes, indicating that induction occurred via independent regulatory pathways.

Nejdl, L., et al. (2015). "Interaction study of arsenic (III and V) ions with metallothionein gene (MT2A) fragment." Int J Biol Macromol 72: 599-605.

Arsenic compounds belong to the most controversial agents concerning human health. Arsenic (As) is considered as a top environmental element influencing human health due to its adverse effects including cancer, diabetes, cardiovascular disease, and reproductive or developmental problems. Despite the proven mutagenic, teratogenic and carcinogenic effects, the arsenic compounds are used for centuries to treat infectious diseases. In our work, we focused on studying of interactions of As(III) and/or As(V) with DNA. Interactions between arsenic ions and DNA were monitored by UV/vis spectrophotometry by measuring absorption and fluorescence spectra, atomic absorption spectrometry, electrochemical measurements (square wave voltammetry) and agarose gel electrophoresis. Using these methods, we observed a stable structure of DNA with As(III) within the concentration range 0.4-6.25 mug mL(-1). Higher As(III) concentration caused degradation of DNA. However, similar effects were not observed for As(V).

Nemer, M., et al. (1995). "Spatial regulation of SpMTA metallothionein gene expression in sea urchin embryos by a regulatory cassette in intron 1." Mech Dev 50(2-3):

131-137.

The SpMTA metallothionein (MT) gene of the sea urchin Strongylocentrotus purpuratus is restricted in its expression to the aboral ectoderm in gastrulae and pluteus larvae. The proximal 1.6 kb of the 5'-flanking region together with the 1.12-kb first intron of the SpMTA gene are sufficient for its correct cell-type specific expression in transgenic embryos. This restricted spatial expression is largely eliminated by deletion of an interior 405-bp region in the intron. Within this region is a 295-bp, genomically repetitive, transposon-like segment (Nemer et al., 1993), containing several sequence motifs highly homologous to posited regulatory elements in the promoters of other genes (Thiebaud et al., 1990). The P3A and P5 sites in this apparent regulatory cassette were shown through competition to bind with relatively high affinities the same nuclear factors, bound by their counterpart sites in the CyIIIa actin promoter.

Nemer, M., et al. (1984). "Developmental regulation, induction, and embryonic tissue specificity of sea urchin metallothionein gene expression." Dev Biol 102(2): 471-482.

Metallothionein (MT) is shown to be present in sea urchin embryos on the basis of its characteristic properties as a small protein (6-7 Da) of extraordinarily high cysteine content, whose biosynthesis is readily induced by heavy metals. Induction by Zn2+ results in the accumulation of the cysteine-rich MT protein, a 0.8 kb MT mRNA and a 2.9 kb nuclear RNA. The amount of MT mRNA is regulated intrinsically through the course of embryogenesis to the pluteus stage: A maternal MT mRNA is poly(A)-deficient and is polyadenylated after fertilization. New MT mRNA begins to accumulate between the seventh and eighth cell cleavage, reaches a maximum at the mesenchyme blastula stage, decreases during gastrulation, and rises again in the early pluteus stage. "Animalizing" embryos with Zn2+ during early embryogenesis causes a sustained accumulation of MT mRNA to levels greater than 25 times the normal amount. MT mRNA is present in high amount in the ectoderm of the pluteus, but is barely detectable in the mesoderm-endoderm tissue fraction. Treatment of either the pluteus or its isolated tissue fractions with Zn2+ results in the induction of MT mRNA accumulation in the mesoderm-endoderm but not in the already MT mRNA-enriched ectoderm. Furthermore, differences in Zn2+ induction of the MT gene in the blastula and gastrula are consistent with a developmental pattern in which MT gene expression is maintained constitutively at a high level in the ectoderm and at a low level in the mesoderm-endoderm tissues, which are, however, preferentially inducible by Zn2+.

Nevrtalova, E., et al. (2014). "Expression response of duplicated metallothionein 3 gene to copper stress in Silene vulgaris ecotypes." Protoplasma 251(6): 1427-1439.

Metallothioneins (MTs) were identified as important players in metal metabolism. MT3 gene presents a key metallothionein controlling copper homeostasis in plants. We have selected one cupricolous and one non-cupricolous ecotype to isolate and analyse the MT3 gene in Silene vulgaris. For expression data comparison, we have also included other metal-tolerant ecotypes. Based on a S. vulgaris BAC library screening, we have identified and sequenced a genomic clone containing MT3 gene (SvMT3). We found that SvMT3 gene has been locally duplicated in a tandem arrangement. Expression analysis and complementation studies using yeast mutants showed that both copies of the SvMT3 gene were functional. Moreover, we examined the expression of MT3 gene(s) in selected ecotypes under different copper treatments to show the tissue-specific expression response to copper stress. We demonstrated that higher copper concentrations specifically affected MT3 expression among ecotypes. Our analysis shows that MT3a has similar expression pattern in cupricolous ecotypes while MT3b has common expression features shared by all metallophyte S. vulgaris ecotypes. Our data indicate that down-regulation of MT3b root expression in higher copper concentrations is associated with copper stress. We propose that there might be a specific regulation of SvMT3s transcription depending on the type of heavy metal tolerance.

Nguyen, A., et al. (2000). "In vivo gene expression profile analysis of metallothionein in renal cell carcinoma." Cancer Lett 160(2): 133-140.

The antiapoptotic and mitogenic responses of metallothionein (MT) have been well documented in vitro. While MT protein overexpression, frequently encountered in a number of human primary tumors, has been shown to be correlated with disease progression, little information is available on the in vivo isoform expression of MT. In this study we have demonstrated the occurrence of MT proteins and further defined their differential expression profile in human primary renal cell carcinoma (RCC). Pooled normal human kidney RNA and paired biopsy specimens (tumor and control) obtained from 11 patients diagnosed with RCC with tumor grade ranging from 1-3 and a pathological staging of T2-T3 (N0M0) were used for the study. Samples were analyzed for the presence of MT protein using immunohistochemical (IHC) analysis and for MT isoform-specific mRNA expression by reverse transcriptase polymerase chain reaction. Metallothionein protein assumed both cytoplasmic and nuclear staining in cancer cells and was detected in eight of 11 samples (72%) with polyclonal antibodies. The immunoreactivity of MT protein, but not its cellular localization, in RCC specimens suggests a

relationship between and advanced disease. While alterations in the basal level of expression of MT-1E, MT-1F and MT-1X genes remained unchanged, significant up-regulation of MT-2A and down-regulation of MT-1A and MT-1G transcripts was observed in RCC tissue specimens when compared with controls. Intriguingly, the paired RCC biopsy specimens had lower MT-1H transcripts than pooled normal human controls. We here provide the first report of the differential expression of MT isoforms in human RCC and that this data further support the role of MT-2A in tumorigenesis.

Niederwanger, M., et al. (2017). "Biomphalaria glabrata Metallothionein: Lacking Metal Specificity of the Protein and Missing Gene Upregulation Suggest Metal Sequestration by Exchange Instead of through Selective Binding." Int J Mol Sci 18(7).

The wild-type metallothionein (MT) of the freshwater snail Biomphalaria glabrata and a natural allelic mutant of it in which a lysine residue was replaced by an asparagine residue, were recombinantly expressed and analyzed for their metal-binding features with respect to Cd(2+), Zn(2+) and Cu(+), applying spectroscopic and mass-spectrometric methods. In addition, the upregulation of the Biomphalaria glabrataMT gene was assessed by quantitative real-time detection PCR. The two recombinant proteins revealed to be very similar in most of their metal binding features. They lacked a clear metal-binding preference for any of the three metal ions assayed-which, to this degree, is clearly unprecedented in the world of Gastropoda MTs. There were, however, slight differences in copper-binding abilities between the two allelic variants. Overall, the missing metal specificity of the two recombinant MTs goes hand in hand with lacking upregulation of the respective MT gene. This suggests that in vivo, the Biomphalaria glabrata MT may be more important for metal replacement reactions through a constitutively abundant form, rather than for metal sequestration by high binding specificity. There are indications that the MT of Biomphalaria glabrata may share its unspecific features with MTs from other freshwater snails of the Hygrophila family.

Niederwanger, M., et al. (2017). "Challenging the Metallothionein (MT) Gene of Biomphalaria glabrata: Unexpected Response Patterns Due to Cadmium Exposure and Temperature Stress." Int J Mol Sci 18(8).

Metallothioneins (MTs) are low-molecular-mass, cysteine-rich, metal binding proteins. In most animal species, they are involved in metal homeostasis and detoxification, and provide protection from oxidative stress. Gastropod MTs are highly diversified, exhibiting unique features and adaptations like metal specificity and multiplications of their metal binding domains. Here, we show that the MT gene of Biomphalaria glabrata, one of the largest MT genes identified so far, is composed in a unique way. The encoding for an MT protein has a three-domain structure and a C-terminal, Cys-rich extension. Using a bioinformatic approach involving structural and in silico analysis of putative transcription factor binding sites (TFBs), we found that this MT gene consists of five exons and four introns. It exhibits a regulatory promoter region containing three metal-responsive elements (MREs) and several TFBs with putative involvement in environmental stress response, and regulation of gene expression. Quantitative real-time polymerase chain reaction (qRT-PCR) data indicate that the MT gene is not inducible by cadmium (Cd) nor by temperature challenges (heat and cold), despite significant Cd uptake within the midgut gland and the high Cd tolerance of metal-exposed snails.

Nishimura, N., et al. (1996). "Evidence for developmentally regulated transcriptional, translational and post-translational control of metallothionein gene expression in hair follicles." Reprod Fertil Dev 8(7): 1089-1096.

The distribution of metallothionein (MT) and MT mRNAs was examined in hair (wool) follicles, where high levels of cell proliferation are found and where the resulting cells provide a temporal record of differentiation events. MT was found in the cytoplasm and some nuclei of follicle bulb cells of the proliferative zone, outer root sheath cells and in basal layer cells of sebaceous glands and sweat glands. The population of 5-bromo-2'-deoxyuridine (BrdU)+ cells in these tissues overlapped, but were not completely coincident with the distribution of MT staining. MT mRNA expression in hair (wool) follicles was assessed by in situ hybridization with four gene-specific sheep MT (sMT) isoforms. Intense signals were obtained with the sMT-Ib probe in follicle bulb cells from the proliferative zone to the keratogenous zone. Signals from the sMT-Ia probe were present in the same cells, but were much weaker. No signals were detected using the sMT-Ic and sMT-II gene-specific probes. The findings suggest that: (1) MT is important in cell proliferation and/or cell differentiation in the hair follicle bulb; (2) MT translation is inhibited during cell differentiation and migration; and (3) tissue-specific expression of uncharacterized sMT isoforms is likely.

Nobeyama, Y. and H. Nakagawa (2017). "Silencing of metallothionein 1A gene in melanoma." J Dermatol Sci 88(2): 232-237.

BACKGROUND: When a CpG island (CGI; a dense cluster of CpGs) located in the 5' region of a gene is methylated, its transcription is suppressed. Tumorigenesis of melanoma is associated with trace elements. Metallothionein 1A is closely associated with the metabolism of trace elements. However, little is known about the metallothionein 1A gene (MT1A) in melanoma. OBJECTIVE: The purpose is to reveal the

methylation and expression status of MT1A in melanoma. METHODS: Quantitative real-time methylation-specific PCR (RT-MSP) and bisulfite sequencing were performed to examine MT1A methylation status. Quantitative real-time reverse transcription-PCR (RT-PCR) was performed to examine MT1A expression. RESULTS: Some melanoma cell lines exhibited high methylation levels of the CGI located in the 5' region of MT1A (5' MT1A CGI) with suppression of MT1A. Other melanoma cell lines and normal cultured melanocytes exhibited low methylation levels of 5' MT1A CGI with expression of MT1A. Treatment with a demethylating agent resulted in transcriptional induction of MT1A in the melanoma cell lines SK-MEL-5 and G-361 with high methylation levels prior to treatment. The methylation levels of 5' MT1A CGI ranged widely from 0.0% to 91.4% in 21 clinical melanoma samples but showed a narrow, low range from 0.0% to 6.4% in 23 clinical melanocytic nevus samples. Data of bisulfite sequencing was generally compatible with those of RT-MSP. The methylation levels ranged according to the types of melanoma (Kruskal-Wallis test, P=0.047). CONCLUSION: MT1A is aberrantly silenced by DNA methylation of 5' MT1A CGI in melanoma.

Obertello, M., et al. (2007). "Functional analysis of the metallothionein gene cgMT1 isolated from the actinorhizal tree Casuarina glauca." Mol Plant Microbe Interact 20(10): 1231-1240.

cgMT1 is a metallothionein (MT)-like gene that was isolated from a cDNA library of young nitrogen-fixing nodules resulting from the symbiotic interaction between Frankia spp. and the actinorhizal tree Casuarina glauca. cgMT1 is highly transcribed in the lateral roots and nitrogen-fixing cells of actinorhizal nodules; it encodes a class I type 1 MT. To obtain insight into the function of cgMT1, we studied factors regulating the expression of the MT promoter region (PcgMT1) using a beta-glucuronidase (gus) fusion approach in transgenic plants of Arabidopsis thaliana. We found that copper, zinc, and cadmium ions had no significant effect on the regulation of PcgMT1-gus expression whereas wounding and H2O2 treatments led to an increase in reporter gene activity in transgenic leaves. Strong PcgMT1-gus expression also was observed when transgenic plants were inoculated with a virulent strain of the bacterial pathogen Xanthomonas campestris pv. campestris. Transgenic Arabidopsis plants expressing cgMT1 under the control of the constitutive 35S promoter were characterized by reduced accumulation of H2O2 when leaves were wounded and by increased susceptibility to the bacterial pathogen X. campestris. These results suggest that cgMT1 could play a role during the oxidative response linked to biotic and abiotic stresses.

Oguro, T. and T. Yoshida (2001). "Effect of ultraviolet A on ornithine decarboxylase and metallothionein gene expression in mouse skin." Photodermatol Photoimmunol Photomed 17(2): 71-78.

BACKGROUND: Ultraviolet A (UVA) is known to induce the expression of many stress responsive genes due to the generation of reactive oxygen species (ROS). However, UVA's role in inducing metallothionein (MT) gene expression has not been studied. Furthermore, our group demonstrated that UVA enhanced 12-O-tetradecanoylphorbol-13-acetate (TPA)-mediated induction of ornithine decarboxylase (ODC) activity in mouse skin (1). METHODS: We examined the interaction of UVA, TPA and antioxidants on the induction of MT and ODC mRNA in mouse skin. Female CD-1 mice were exposed to UVA (19 J/cm2) and total RNA was isolated from the skin. Northern blot analysis for MT and ODC mRNAs was performed. ODC activity in mouse epidermis was also determined in some experiments. RESULTS: UVA induced MT mRNA in mouse skin; however, it did not increase ODC mRNA. 1,4-Diazabicylo-[2,2,2]-octane (DABCO), a singlet oxygen scavenger, reduced UVA-mediated induction of MT mRNA by 40%. The data suggest that ROS produced by UVA exposure may contribute to its ability to induce MT mRNA. UVA slightly enhanced TPA-mediated ODC mRNA induction, while it enhanced ODC enzyme activity 70%. UVA additively intensified TPA-mediated MT mRNA induction. alpha-Tocopherol pretreatment inhibited the induction of ODC enzyme activity by TPA treatment combined with UVA exposure (TPA + UVA); however, alpha-tocopherol had less of an inhibitory effect on ODC mRNA induction by TPA + UVA. Curcumin, a plant pigment, dramatically inhibited both TPA- and TPA + UVA-induced expression of ODC and MT genes. CONCLUSIONS: These results demonstrate that UVA can induce MT gene expression and enhance TPA-induced ODC and MT gene expression. The data further suggest that these effects are partially mediated by ROS.

Ohta, H. (1996). "[Role of trace elements on metallothionein gene expression]." Nihon Rinsho 54(1): 40-45.

Metallothionein (MT) is low-molecular-weight metal binding proteins with a high cysteine content, which provide the protection against metal toxicity and the regulation of essential trace elements such as zinc and copper. MT is inducible proteins by various metals, hormones, cytokines, and endogenous and exogenous agents. Human MT genes locate on chromosome 16, and mainly three isoforms of MT (MT-I, MT-II, MT-III) have been identified. MT genes expression is controlled mainly at the transcriptional level, and is regulated by the interaction between cis-acting elements (MRE, GRE, IRE) and trans-acting factors (MTF-1, ZRF, MRE-BF-1 and 2, MREBP).

Regulation of MT genes by metals may be mediated by MTF-1 interacting with MREs and zinc functions to release MTF-1 from an inhibitor (MTI).

Okumoto, D. S. and V. A. Bohr (1987). "DNA repair in the metallothionein gene increases with transcriptional activation." Nucleic Acids Res 15(23): 10021-10030.

We have studied DNA repair in the Chinese Hamster Ovary (CHO) metallothionein (MT) gene after UV-light induced damage. The repair was examined comparatively with or without transcriptional activation of the gene by incubation in the presence of the heavy metal ZnCl2. Whereas the repair efficiency was very low in the uninduced state, it increased significantly after induction of the gene. The presence of ZnCl2 did not appear to change other repair parameters in the cells. The overall genome DNA repair efficiency after UV irradiation was similar whether or not the gene was induced and the preferential DNA repair pattern in the essential dihydrofolate reductase (DHFR) gene which we have previously described was unaffected by the presence of ZnCl2. Based upon repair analysis in two different restriction fragments containing the MT I gene, we conclude that the region of efficient repair after induction is considerably larger than the 1 kb size of the gene. The results suggest that the accessibility of a genomic region to DNA repair enzymes may be regulated by the local chromatin structure in a dynamic manner.

Olsson, P. E., et al. (1990). "Differences in metallothionein gene expression in primary cultures of rainbow trout hepatocytes and the RTH-149 cell line." Biochim Biophys Acta 1049(1): 78-82.

Primary cultures of rainbow trout, Salmo gairdneri, hepatocytes were used to study the expression of metallothionein (MT) genes in response to steroid hormone treatment. The expression pattern was compared to that of an immortal cell line (RTH-149). MT mRNA accumulated in both cell cultures after exposure to zinc while 17 beta-oestradiol had no effect in either system. Treatment with cortisol and corticosterone resulted in a 2-fold increase of metallothionein mRNA levels in the primary cultures but had no effect in the RTH-149 cell culture. Primary cultures that were exposed to zinc or cortisol showed a high temporal correlation (r = 0.974) between MT mRNA and MT protein levels. The basal level expression was 3-4-fold higher in primary cultures than in RTH-149 cells. The present study demonstrates the inducibility of rainbow trout MT genes in response to glucocorticoids. It further indicates that primary cultures are to be preferred to immortal cell lines when investigating the inducibility of MT mRNA.

Olsson, P. E., et al. (1995). "Structural and functional analysis of the rainbow trout (Oncorhyncus mykiss) metallothionein-A gene." Eur J Biochem 230(1): 344-349.

In the present study, the distal part of the 5'-flanking region of the rainbow trout metallothionein-A promoter was sequenced in order to identify cis-acting regulatory elements. Analysis of this sequence combined with that previously reported for the 5'-flanking region directly proximal to the start of transcription revealed several putative regulatory sequences. In total, six metal-responsive elements (MREs) were identified; these sequences were organised into two clusters, one containing two copies of MRE and located close to the predicted TATA box sequence, and a second consisting of four MREs and lying 500-700 bp upstream from the start of transcription. In addition, the 5'-flanking region contained sequences sharing high similarity with the activator protein 1 consensus sequence as well as one nuclear-factor-interleukin-6-responsive element. Functional analysis of the promoter was performed by introducing deletion mutants of the 5'-flanking region into the vector pGL-2, directly upstream from the luciferase reporter gene. Both MRE clusters were needed for maximal metal inducibility in both rainbow trout hepatoma (RTH-149) and human hepatoblastoma (Hep G2) cell lines. Furthermore, the distal region was found to be functional in promoting gene transcription following exposure of RTH-149 cells to hydrogen peroxide.

Orian, J. M., et al. (1989). "The expression of a metallothionein-ovine growth hormone fusion gene in transgenic mice does not impair fertility but results in pathological lesions in the liver." Endocrinology 124(1): 455-463.

The physiological effects of high serum levels of ovine GH (oGH) were studied in three generations of transgenic mice carrying a metallothionein 1-(MT)oGH fusion gene. Livers of mice expressing oGH were enlarged, irrespective of the level of serum oGH detected. In mice expressing high levels of oGH, direct measurements of hepatocytes in liver sections revealed that cell and nuclear size were abnormally large. Hepatocytes of different transgenic mice varied from 1.4-2.2 times normal size and hepatocyte nuclei varied from 1.7-2.4 times normal size. In addition, intranuclear inclusions were observed in hepatocytes of transgenic mice and their presence was always associated with high serum levels of oGH. In contrast to female transgenic mice containing mouse MT-human, rat, or bovine GH fusion genes female mice containing the MT oGH fusion gene were fertile and their pituitary glands showed synthesis of GH.

Orian, J. M., et al. (1990). "New murine model for hepatocellular carcinoma: transgenic mice expressing metallothionein-ovine growth hormone fusion gene." J Natl Cancer Inst 82(5): 393-398.

Hepatocellular carcinomas developed at a

high frequency in the livers of transgenic (C57BL/6 X SJL/J)F1 mice under the influence of growth hormone. Three lines of giant transgenic mice expressing a mouse metallothionein-ovine growth hormone fusion gene were generated. The giant mice weighed twice as much as control littermates. The three lines of giant mice expressing very high levels of growth hormone were bred over several generations. Mice from all three lines developed hepatocellular tumors, including adenoma and carcinoma. The occurrence of tumors was age-dependent, and their incidence increased to 70% of the mice studied after 43 weeks of age. Pathologic changes in the livers resembled those observed in rats in which hepatocellular carcinomas are induced chemically. Transgenic mice carrying the metallothionein-ovine growth hormone fusion gene represent a new model for hepatocellular carcinogenesis. This model exemplifies the oncogenic potential for a sustained proliferative growth stimulus within an organ.

Otsuka, F., et al. (1994). "Purification and characterization of a protein that binds to metal responsive elements of the human metallothionein IIA gene." J Biol Chem 269(38): 23700-23707.

Metal responsive element (MRE) is a cis-acting DNA motif located in the upstream region of vertebrate metallothionein genes, which can confer metal responsiveness on downstream heterologous promoters. A protein that binds to the MRE sequence in a zinc-dependent manner (zinc regulatory factor; ZRF) was purified 16,000-fold from HeLa cell nuclear extracts by means of the avidin-biotin method, in which a complex formed between ZRF and a biotinylated probe containing MRE was trapped by streptavidin-agarose beads, and ZRF was recovered by salt extraction. By repeating the method three times, a homogeneous 116-kDa protein was obtained whose recovery was zinc-dependent and MRE sequence-specific. UV cross-linking analysis also revealed that a protein that specifically binds to MRE has the same molecular mass as the purified protein. Zinc-dependent and MRE sequence-specific footprints of ZRF were obtained on MREa and MREb in the upstream region of the human metallothionein IIA gene. The ZRF-MRE complex dissociates by the addition of chelating reagents, suggesting a direct role of zinc ions in the DNA binding of ZRF. Partial amino acid sequences of ZRF were found to be highly homologous to those of a mouse MRE-binding protein, mMTF-1.

Otsuka, F., et al. (2007). "[Mechanism of metallothionein gene activation mediated by heavy-metal dependent transcription factor MTF-1]." Yakugaku Zasshi 127(4): 675-684.

Transcriptional activation of metallothionein (MT) genes by heavy metals is a valuable system for understanding the functions of MT as well as the cellular response against heavy metals. Although it is now known that heavy metal signals culminating in MT induction converge upon a transcription factor MTF-1, the mechanism underlying the MTF-1 response to heavy metals has not been elucidated. To address this issue, we investigated various aspects of the in vivo response of MTF-1 against heavy metals. Chromatin immunoprecipitation assay showed that heavy metal-dependent DNA binding of MTF-1 is the critical step in vivo. MTF-1 is primarily localized in the nucleus so that heavy metal-dependent nuclear translocation demonstrated by other groups does not seem to be universal and hence may not be critical for activation of MTF-1. In the six Zn finger motifs, the hallmark of MTF-1, the third and the fourth fingers are essential for the nuclear localization of MTF-1. Furthermore, all fingers except the last are important for transcriptional activation function of MTF-1, suggesting their key role for MTF-1 function. Also, a cysteine cluster structure located in the C-terminal region of MTF-1 is critical for transactivating function of MTF-1. These results suggest a central role of the Zn-finger domain and intramolecular cooperation through a structural change of MTF-1 for its response to heavy metal challenge.

Otsuka, F., et al. (1993). "A human metal responsive element-binding protein interacts with a homologous element of the mouse metallothionein-I gene." Ind Health 31(4): 133-142.

Metallothionein (MT) is thought to play a central role in the detoxification of heavy metals, and thus studies on its regulation are toxicologically important. Heavy metal-dependent induction of MT genes is mediated by metal responsive elements (MREs) located upstream of the genes. Zinc regulatory factor (ZRF) is a zinc-dependent MRE-binding protein that was originally detected in HeLa cell nuclear extracts using the most proximal MRE of the human MT-IIA gene (hMREa) as a probe. We show that ZRF in HeLa cell nuclear extracts can also bind to the most potent MRE of the mouse MT-I gene (mMREd). This finding was further confirmed by using partially purified ZRF. Moreover, cadmium could not promote complex formation between ZRF and mMREd at any concentration tested, as is also the case with ZRF and hMREa. These observations suggest that the transcriptional regulatory system of MT genes by zinc is conserved beyond species.

Otsuka, T., et al. (2013). "Suppression of metallothionein 3 gene expression by androgen in LNCaP prostate cancer cells." Biomed Rep 1(4): 614-618.

Androgen deprivation therapy is the standard treatment for prostate cancer. However, tumors often progress towards a more aggressive phenotype despite treatment. Prostate tissue has a high zinc concentration,

which may correlate with prostate cancer progression. Therefore, we investigated the effect of dihydrotestosterone (DHT) on the gene expression of metallothioneins (MTs) and zinc transporters in prostate cancer with quantitative real-time polymerase chain reaction (PCR). The MT3 gene expression in LNCaP cells was suppressed by DHT in a dose-dependent manner. However, it increased in a culture medium containing androgen-deficient charcoal-stripped fetal bovine serum (FBS). Bicalutamide, an androgen receptor antagonist, increased the gene expression of MT3 and partially reversed the suppression of MT3 gene expression induced by DHT. In PC-3 cells lacking androgen receptors, DHT and bicalutamide exerted no effect on MT3 gene expression. The reporter gene assay with a luciferase reporter plasmid containing the 5'-flanking region of MT3 demonstrated a decrease in luciferase activity caused by DHT that was reversed by bicalutamide. These results suggest that MT3 gene expression is downregulated by androgen.

Otto, E., et al. (1987). "A DNA segment controlling metal-regulated expression of the Drosophila melanogaster metallothionein gene Mtn." Mol Cell Biol 7(5): 1710-1715.

Cloned fragments of DNA including the Drosophila melanogaster metallothionein gene Mtn and different amounts of 5' flanking sequences were introduced into flies by P-element-mediated germ line transformation. Comparison of RNA levels in different transformants revealed that metal-regulated and tissue-specific expression of Mtn requires no more than 373 base pairs upstream of the initiation site of transcription. Transformants having an additional, transcribed copy of Mtn could tolerate increased concentrations of cadmium, indicating that Mtn expression is directly related to this phenotype. In separate experiments, these D. melanogaster promoter sequences were fused to the coding sequences of the herpes simplex virus thymidine kinase (TK) gene. After transfection of this fusion into baby hamster kidney cells, increases in TK activity and accumulation of TK RNA were inducible by metals. A series of 5' and 3' deletions showed that D. melanogaster sequences from -130 to -6 were sufficient to confer metal-regulated expression to the TK gene. The function of the D. melanogaster metallothionein promoter in mammalian cells indicates that the mechanism controlling metal regulation is evolutionarily conserved.

Otto, E., et al. (1986). "Structure and expression of a tandem duplication of the Drosophila metallothionein gene." Proc Natl Acad Sci U S A 83(16): 6025-6029.

A strain of cadmium-resistant Drosophila containing a chromosomal duplication of the metallothionein gene was isolated. This duplication is stably inherited in the absence of selective pressure, and larvae homozygous for it can produce approximately twice as much metallothionein RNA as wild-type larvae. The entire duplication was cloned within a 5.7-kilobase fragment; this fragment contained a direct, tandem repeat of 2.2 kilobases of DNA: 228 bases of 5' flanking DNA, the entire transcription unit, and 1.4 kilobases of 3' flanking sequences. The 3' region of the first repeated unit is joined to the 5' region of the second unit by a 6-base-pair segment we define as the novel joint. This joint forms part of a 10-base-pair inverted repeat of a segment within the 3' region of the first unit. Comparison of the sequences of the 5' and 3' boundaries revealed no extensive regions of similarity at a position corresponding to the novel joint, thus suggesting that a mechanism other than homologous recombination was involved in the origin of this duplication.

Pakdee, O., et al. (2019). "Functional characterization of metallothionein-like genes from Physcomitrella patens: expression profiling, yeast heterologous expression, and disruption of PpMT1.2a gene." Planta 250(2): 427-443.

MAIN CONCLUSION: Physcomitrella patens contains four metallothionein-like genes. Three were shown to confer metal tolerance in yeast. Transcript profiling suggests their roles in senescence and reproductive development or cadmium and oxidative stress. Metallothioneins (MTs) have been suggested to play various roles including metal detoxification, nutrient remobilization, ROS scavenging, stress tolerance, and plant development. However, little is known about the forms and functions of MTs in bryophytes. The moss Physcomitrella patens genome was found to contain four MT-like genes. Amino acid sequence composition showed that the P. patens MTs (PpMTs) were clustered with Type 1 plant MTs, and could be further classified into two sub-types, herein referred to as sub-type 1: PpMT1.1a and PpMT1.1b and sub-type 2: PpMT1.2a and PpMT1.2b. Transcript abundance of PpMT1.1b and PpMT1.2b was upregulated in the gametophore compared to protonema, and all, except PpMT1.2a, were highly induced in senescing gametophytes. PpMT1.1a and PpMT1.1b transcripts were upregulated in protonema treated with cadmium and hydrogen peroxide. Unlike many higher plant MTs, the PpMT transcript abundance was not strongly induced in response to copper and zinc. These results suggest that PpMTs may play a role in protecting P. patens from cadmium and oxidative stress and may be involved in tissues senescence and reproductive development. The PpMTs, except PpMT1.2b, were also able to confer metal tolerance and accumulation when heterologously expressed in the cup1 yeast. A P. patens mutant lacking PpMT1.2a through targeted gene disruption was generated. However, it did not show any alteration in

growth phenotypes under senescence-induced conditions or hypersensitivity to cadmium, copper, zinc, H2O2, and NaCl stresses. Further characterization of additional P. patens mutants lacking single or multiple PpMTs may provide insight into the physiological roles of bryophytic MTs.

Pal, D., et al. (2014). "Metallothionein gene expression in renal cell carcinoma." Indian J Urol 30(3): 241-244.

INTRODUCTION: Metallothioneins (MTs) are a group of low-molecular weight, cysteine-rich proteins. In general, MT is known to modulate three fundamental processes: (1) the release of gaseous mediators such as hydroxyl radical or nitric oxide, (2) apoptosis and (3) the binding and exchange of heavy metals such as zinc, cadmium or copper. Previous studies have shown a positive correlation between the expression of MT with invasion, metastasis and poor prognosis in various cancers. Most of the previous studies primarily used immunohistochemistry to analyze localization of MT in renal cell carcinoma (RCC). No information is available on the gene expression of MT2A isoform in different types and grades of RCC. MATERIALS AND METHODS: In the present study, total RNA was isolated from 38 histopathologically confirmed cases of RCC of different types and grades. Corresponding adjacent normal renal parenchyma was taken as control. Real-time polymerase chain reaction (RT PCR) analysis was done for the MT2A gene expression using beta-actin as an internal control. All statistical calculations were performed using SPSS software. RESULTS: The MT2A gene expression was found to be significantly increased ($P < 0.01$) in clear cell RCC in comparison with the adjacent normal renal parenchyma. The expression of MT2A was two to three-fold higher in sarcomatoid RCC, whereas there was no change in papillary and collecting duct RCC. MT2A gene expression was significantly higher in lower grade (grades I and II, $P < 0.05$), while no change was observed in high-grade tumor (grade III and IV) in comparison to adjacent normal renal tissue. CONCLUSION: The first report of the expression of MT2A in different types and grades of RCC and also these data further support the role of MT2A in tumorigenesis.

Patankar, H. V., et al. (2019). "Overexpression of a Metallothionein 2A Gene from Date Palm Confers Abiotic Stress Tolerance to Yeast and Arabidopsis thaliana." Int J Mol Sci 20(12).

Although the date palm tree is an extremophile with tolerance to drought and certain levels of salinity, the damage caused by extreme salt concentrations in the soil, has created a need to explore stress-responsive traits and decode their mechanisms. Metallothioneins (MTs) are low-molecular-weight cysteine-rich proteins that are known to play a role in decreasing oxidative damage during abiotic stress conditions. Our previous study identified date palm metallothionein 2A (PdMT2A) as a salt-responsive gene, which has been functionally characterized in yeast and Arabidopsis in this study. The recombinant PdMT2A protein produced in Escherichia coli showed high reactivity against the substrate 5'-dithiobis-2-nitrobenzoic acid (DTNB), implying that the protein has the property of scavenging reactive oxygen species (ROS). Heterologous overexpression of PdMT2A in yeast (Saccharomyces cerevisiae) conferred tolerance to drought, salinity and oxidative stresses. The PdMT2A gene was also overexpressed in Arabidopsis, to assess its stress protective function in planta. Compared to the wild-type control, the transgenic plants accumulated less Na(+) and maintained a high K(+)/Na(+) ratio, which could be attributed to the regulatory role of the transgene on transporters such as HKT, as demonstrated by qPCR assay. In addition, transgenic lines exhibited higher chlorophyll content, higher superoxide dismutase (SOD) activity and improved scavenging ability for reactive oxygen species (ROS), coupled with a better survival rate during salt stress conditions. Similarly, the transgenic plants also displayed better drought and oxidative stress tolerance. Collectively, both in vitro and in planta studies revealed a role for PdMT2A in salt, drought, and oxidative stress tolerance.

Paul-Pont, I., et al. (2012). "Cloning, characterization and gene expression of a metallothionein isoform in the edible cockle Cerastoderma edule after cadmium or mercury exposure." Ecotoxicol Environ Saf 75(1): 119-126.

Metallothionein (MT) genes encode crucial metal-binding proteins ubiquitously expressed in living organisms and which play important roles in homeostasis of essential metals and detoxification processes. Here, the molecular organization of the first metallothionein gene of the edible cockle Cerastoderma edule and its expression after cadmium (Cd) or mercury (Hg) exposures were determined. The resulting sequence (Cemt1) exhibits unusual features. The full length cDNA encodes a protein of 73 amino acids with nine classical Cys-X((1-3))-Cys motifs, but also one Cys-Cys not generally found in molluscan MT. Moreover, characterization of the molecular organization of the Cemt1 gene revealed two different alleles (A1 and A2) with length differences due to large deletion events in their intronic sequences involving direct Short Interspersed repeated Elements (SINE), while their exonic sequences were identical. To our knowledge, such large excision mechanisms have never before been reported in a bivalve gene sequence. After 10 days of Cd exposure at environmentally relevant doses, quantitative real-time PCR revealed a strong induction of Cemt1 in gills of C. edule.

Surprisingly, neither induction of the Cemt1 gene nor of MT protein was shown after Hg exposure, despite the fact that this organism is able to bioaccumulate a high amount of this trace metal which is theoretically one of the most powerful inducers of MT biosynthesis.

Pautot, V., et al. (1989). "Expression of a mouse metallothionein gene in transgenic plant tissues." Gene 77(1): 133-140.

Three gene constructions based on a mouse metallothionein I gene (mMT-I) were introduced into tobacco using a Ri plasmid vector system to test the effectiveness of animal gene regulatory signals in plant cells. No transcription from the native mouse gene was observed. In plant cells bearing chimeric mMT-I genes in which transcription was driven by the nopaline synthase promoter, neither polyadenylation nor splicing of mMT-I pre-mRNA was observed. Detailed comparisons of mMT-I sequences with those of known plant genes were carried out; slight differences in regions of known consensus sequences may be at least partly responsible for the non-recognition of mMT-I gene regulatory signals in plant cells, though other as yet unidentified, potentially necessary sequences may also be involved.

Pavlakis, G. N. and D. H. Hamer (1983). "Regulation of a metallothionein-growth hormone hybrid gene in bovine papilloma virus." Proc Natl Acad Sci U S A 80(2): 397-401.

We have constructed bovine papilloma virus recombinants carrying a hybrid gene in which human growth hormone structural sequences are fused to the promoter and presumptive control region of the mouse metallothionein-I gene. Mouse cells transformed with the recombinants synthesize metallothionein-growth hormone hybrid mRNA with the same 5' end as metallothionein mRNA. Hybrid mRNA is inducible by cadmium but not by dexamethasone, whereas the chromosomal metallothionein genes in the same cells are inducible by both agents. This indicates that heavy metals and glucocorticoids regulate the mouse metallothionein-I gene by independent mechanisms. Furthermore, because the transformed cells produce large quantities of human growth hormone polypeptide, it appears that bovine papilloma virus-metallothionein vectors may be useful for obtaining the efficient and regulatable expression of other eukaryotic gene products. We have constructed recombinants that allow the insertion of new structural sequences under the control of the metallothionein promoter.

Pazirandeh, M., et al. (1995). "Expression of the Neurospora crassa metallothionein gene in Escherichia coli and its effect on heavy-metal uptake." Appl Microbiol Biotechnol 43(6): 1112-1117.

The gene coding for the Neurospora crassa metallothionein protein was chemically synthesized and cloned into the fusion expression vectors pMal-c and pMal-p. Cell-fractionation experiments demonstrated the proper localization of the pMal-c and pMal-p- expressed proteins to the cytosol and periplasm of the bacteria respectively. Control bacteria as well as the recombinant bacteria producing the metallothionein protein were incubated with solutions of 109Cd at concentrations of 0.2 microM, 1 microM, and 10 microM. The recombinant bacteria were able to accumulate significantly more 109Cd than control bacteria at all concentrations tested. Cadmium accumulation was rapid and highly selective. Maximum uptake was achieved at a pH of 7.0, with lower accumulation at lower or higher pH values. The pH-dependent uptake of cadmium by the recombinant bacteria was exploited to strip off the bound cadmium from the recombinant bacteria and to regenerate most of the cadmium-binding sites. These observations suggest the potential for using a metallothionein-based biosorbent for certain heavy-metal removal applications.

Peden, K. W., et al. (1989). "Isolation of rat Schwann cell lines: use of SV40 T antigen gene regulated by synthetic metallothionein promoters." Exp Cell Res 185(1): 60-72.

Synthetic promoter elements from the mouse metallothionein-I promoter controlling the expression of SV40 T antigen have been tested for their efficacy in cloning rat Schwann cell lines that retained the characteristic properties of these cells and could be passed in culture indefinitely. The constructed promoters contained either four (MT4) or five (MT5) copies of a metal regulatory element 5' to the CAAT and TATA elements from the HSV-1 thymidine kinase gene. Characterization of these promoters in transient expression assays and transformation assays showed that both MT5 and MT4 were approximately 10-fold and 15-fold, respectively, weaker than the wild-type MT-I promoter in the presence of heavy metal inducer. However, in the absence of inducer, the basal activity of both MT5 and MT4 was barely detectable and much lower than that of MT-I. Schwann cells were transfected with plasmids containing the SV40 T antigen gene under the control of the different metallothionein promoters and cell lines were established from each. Only with the MT5 and MT4 promoters were lines obtained that resembled secondary Schwann cells in culture in their morphology, generation time, and demonstration of contact inhibition. In the presence of zinc, the expression of T antigen in the lines derived with MT5 and MT4 was about 10-fold lower than that derived with MT-I. On removal of the inducer this level was reduced, and in one cell line T antigen was undetectable. Concomitant with the reduction in T antigen expression there was an increased expression of Po, a protein specific to myelin-forming Schwann cells, and a decreased

expression of glial fibrillary acidic protein, a protein expressed only in nonmyelin-forming Schwann cells. These cell lines, therefore, closely resemble untransfected Schwann cells in culture.

Pedrini-Martha, V., et al. (2016). "Physiological, Diurnal and Stress-Related Variability of Cadmium-Metallothionein Gene Expression in Land Snails." PLoS One 11(3): e0150442.

The terrestrial Roman snail Helix pomatia has successfully adapted to strongly fluctuating conditions in its natural soil habitat. Part of the snail's stress defense strategy is its ability to express Metallothioneins (MTs). These are multifunctional, cysteine-rich proteins that bind and inactivate transition metal ions (Cd(2+), Zn(2+), Cu(+)) with high affinity. In Helix pomatia a Cadmium (Cd)-selective, inducible Metallothionein Isoform (CdMT) is mainly involved in detoxification of this harmful metal. In addition, the snail CdMT has been shown to also respond to certain physiological stressors. The aim of the present study was to investigate the physiological and diurnal variability of CdMT gene expression in snails exposed to Cd and non-metallic stressors such as desiccation and oxygen depletion. CdMT gene expression was upregulated by Cd exposure and desiccation, whereas no significant impact on the expression of CdMT was measured due to oxygen depletion. Overall, Cd was clearly more effective as an inducer of the CdMT gene expression compared to the applied non-metallic stressors. In unexposed snails, diurnal rhythmicity of CdMT gene expression was observed with higher mRNA concentrations at night compared to daytime. This rhythmicity was severely disrupted in Cd-exposed snails which exhibited highest CdMT gene transcription rates in the morning. Apart from diurnal rhythmicity, feeding activity also had a strong impact on CdMT gene expression. Although underlying mechanisms are not completely understood, it is clear that factors increasing MT expression variability have to be considered when using MT mRNA quantification as a biomarker for environmental stressors.

Peng, D., et al. (2011). "Location-specific epigenetic regulation of the metallothionein 3 gene in esophageal adenocarcinomas." PLoS One 6(7): e22009.

BACKGROUND: Metallothionein 3 (MT3) maintains intracellular metal homeostasis and protects against reactive oxygen species (ROS)-induced DNA damage. In this study, we investigated the epigenetic alterations and gene expression of the MT3 gene in esophageal adenocarcinomas (EACs). METHODS AND RESULTS: Using quantitative bisulfite pyrosequencing, we detected unique DNA methylation profiles in the MT3 promoter region. The CpG nucleotides from -372 to -306 from the transcription start site (TSS) were highly methylated in tumor (n = 64) and normal samples (n = 51), whereas CpG nucleotides closest to the TSS (-4 and +3) remained unmethylated in all normal and most tumor samples. Conversely, CpG nucleotides in two regions (from -139 to -49 and +296 to +344) were significantly hypermethylated in EACs as compared to normal samples [FDR<0.001, -log10(FDR)>3.0]. The DNA methylation levels from -127 to -8 CpG sites showed the strongest correlation with MT3 gene expression (r = -0.4, P<0.0001). Moreover, the DNA hypermethylation from -127 to -8 CpG sites significantly correlated with advanced tumor stages and lymph node metastasis (P = 0.005 and P = 0.0313, respectively). The ChIP analysis demonstrated a more repressive histone modification (H3K9me2) and less active histone modifications (H3K4me2, H3K9ace) in OE33 cells than in FLO-1 cells; concordant with the presence of higher DNA methylation levels and silencing of MT3 expression in OE33 as compared to FLO-1 cells. Treatment of OE33 cells with 5-Aza-deoxycitidine restored MT3 expression with demethylation of its promoter region and reversal of the histone modifications towards active histone marks. CONCLUSION: In summary, EACs are characterized by frequent epigenetic silencing of MT3. The choice of specific regions in the CpG island is a critical step in determining the functional role and prognostic value of DNA methylation in cancer cells.

Peng, X. D., et al. (2001). "The growth hormone (GH)-axis of GH receptor/binding protein gene-disrupted and metallothionein-human GH-releasing hormone transgenic mice: hypothalamic neuropeptide and pituitary receptor expression in the absence and presence of GH feedback." Endocrinology 142(3): 1117-1123.

Elevation of circulating GH acts to feed back at the level of the hypothalamus to decrease GH-releasing hormone (GHRH) and increase somatostatin (SRIF) production. In the rat, GH-induced changes in GHRH and SRIF expression are associated with changes in pituitary GHRH receptor (GHRH-R), GH secretagogue receptor (GHS-R), and SRIF receptor subtype messenger RNA (mRNA) levels. These observations suggest that GH regulates its own synthesis and release not only by altering expression of key hypothalamic neuropeptides but also by modulating the sensitivity of the pituitary to hypothalamic input, by regulating pituitary receptor synthesis. To further explore this possibility, we examined the relationship between the expression of hypothalamic neuropeptides [GHRH, SRIF, and neuropeptide Y (NPY)] and pituitary receptors [GHRH-R, GHS-R, and SRIF receptor subtypes (sst2 and sst5)] in two mouse strains with alterations in the GH-axis; the GH receptor/binding protein gene-disrupted mouse (GHR/BP-/-) and the metallothionein promoter driven human GHRH (MT-hGHRH)

transgenic mouse. In GHR/BP-/- mice, serum insulin-like growth factor I levels are low, and circulating GH is elevated because of the lack of GH negative feedback. Hypothalamic GHRH mRNA levels in GHR/BP-/- mice were 232 +/- 20% of GHR/BP+/+ littermates ($P < 0.01$), whereas SRIF and NPY mRNA levels were reduced to 86 +/- 2% and 52 +/- 3% of controls, respectively ($P < 0.05$; ribonuclease protection assay). Pituitary GHRH-R and GHS-R mRNA levels of GHR/BP-/- mice were elevated to 275 +/- 55% and 319 +/- 68% of GHR/BP+/+ values ($P < 0.05$, respectively), whereas the sst2 and sst5 mRNA levels did not differ from GHR/BP intact controls as determined by multiplex RT-PCR. Therefore, in the absence of GH negative feedback, both hypothalamic and pituitary expression is altered to favor stimulation of GH synthesis and release. In MT-hGHRH mice, ectopic hGHRH transgene expression elevates circulating GH and insulin-like growth factor I. In this model of GH excess, endogenous (mouse) hypothalamic GHRH mRNA levels were reduced to 69 +/- 6% of nontransgenic controls, whereas SRIF mRNA levels were increased to 128 +/- 6% ($P < 0.01$). NPY mRNA levels were not significantly affected by hGHRH transgene expression. Also, MT-hGHRH pituitary GHRH-R and GHS-R mRNA levels did not differ from controls. However, sst2 and sst5 mRNA levels in MT-hGHRH mice were increased to 147 +/- 18% and 143 +/- 16% of normal values, respectively ($P < 0.05$). Therefore, in the presence of GH negative feedback, both hypothalamic and pituitary expression is altered to favor suppression of GH synthesis and release.

Penkowa, M., et al. (2006). "Novel roles for metallothionein-I + II (MT-I + II) in defense responses, neurogenesis, and tissue restoration after traumatic brain injury: insights from global gene expression profiling in wild-type and MT-I + II knockout mice." J Neurosci Res 84(7): 1452-1474.

Traumatic injury to the brain is one of the leading causes of injury-related death or disability, especially among young people. Inflammatory processes and oxidative stress likely underlie much of the damage elicited by injury, but the full repertoire of responses involved is not well known. A genomic approach, such as the use of microarrays, provides much insight in this regard, especially if combined with the use of gene-targeted animals. We report here the results of one of these studies comparing wild-type and metallothionein-I + II knockout mice subjected to a cryolesion of the somatosensorial cortex and killed at 0, 1, 4, 8, and 16 days postlesion (dpl) using Affymetrix genechips/oligonucleotide arrays interrogating approximately 10,000 different murine genes (MG_U74Av2). Hierarchical clustering analysis of these genes readily shows an orderly pattern of gene responses at specific times consistent with the processes involved in the initial tissue injury and later regeneration of the parenchyma, as well as a prominent effect of MT-I + II deficiency. The results thoroughly confirmed the importance of the antioxidant proteins MT-I + II in the response of the brain to injury and opened new avenues that were confirmed by immunohistochemistry. Data in KO, MT-I-overexpressing, and MT-II-injected mice strongly suggest a role of these proteins in postlesional activation of neural stem cells.

Peterson, M. G., et al. (1988). "The sheep metallothionein gene family. Structure, sequence and evolutionary relationship of five linked genes." Eur J Biochem 174(2): 417-424.

Southern blot analysis of the sheep genome revealed a metallothionein gene family with at least nine members. Two overlapping cosmid clones spanning approximately 67 kb and containing five metallothionein genes have been isolated. DNA sequence analysis reveals that one of these is a metallothionein II variant, three are metallothionein I variants and one is a truncated metallothionein pseudogene containing only the first exon. The predicted amino acid sequence was compared with previously reported amino acid composition data of sheep metallothioneins [Whanger, P. D., Oh, S.-H. & Deagen, J. T. (1981) J. Nutr. 111, 1207-1215], and this suggests that we have isolated the genes encoding the major protein isoforms found in the sheep liver. The promoter regions of these genes contain many conserved elements, among them metal-regulatory elements and putative glucocorticoid-responsive elements. However, there are a number of differences between these genes in the arrangement of these elements. Sequence comparisons indicate that the multiple metallothionein I genes and the pseudogene appear to have resulted from sequential duplication events, and a larger cluster of metallothionein I genes may have been disrupted leading to the formation of the pseudogene.

Peterson, M. G. and J. F. Mercer (1986). "Structure and regulation of the sheep metallothionein-Ia gene." Eur J Biochem 160(3): 579-585.

Screening of a sheep genomic lambda library with a sheep metallothionein-I cDNA clone resulted in the isolation of a 13,200-base-pair fragment containing a metallothionein gene which DNA sequence analysis identified as the gene encoding the cloned cDNA. The two introns occur at identical positions to those in other mammalian metallothioneins but are considerably larger. The first intron contains a DNA element that is present in a related but not identical form in many places in the sheep genome. Comparison of the promoter sequences of this gene (sMT-Ia) with the promoters of metallothionein genes from other species

identified a number of conserved regions which may be important in the regulation of this gene by heavy metals, glucocorticoids and alpha-interferon. In sheep fibroblasts, the levels of sMT-Ia mRNA was found to be maximally elevated (95-fold) in the presence of zinc or cadmium and elevated 30-fold in the presence of copper. Dexamethasone had no effect upon mRNA levels. Thus this gene shows a pattern of regulation similar to the human MT-If gene, but distinct from the other human and mouse metallothionein genes so far reported.

Philcox, J. C., et al. (1995). "Endotoxin-induced inflammation does not cause hepatic zinc accumulation in mice lacking metallothionein gene expression." Biochem J 308 (Pt 2): 543-546.

The action of endotoxin lipopolysaccharide (LPS) on hepatic Zn uptake was examined in mice lacking expression of metallothionein (MT)-1 and MT-II genes. Hepatic Zn concentrations, which in normal control mice increased by a mean 29% (MT elevated 20-fold) 16 h post-LPS exposure, did not increase in MT-null mice. Plasma Zn fell by 68% in controls and 32% in MT-null mice. The time course of LPS action in normal mice was characterized by a rapid reduction (-74% at 4 h, -81% at 8 h) and partial recovery (-39% at 24 h) in plasma Zn, with a progressive increase over 24 h in hepatic concentrations of MT (by 36-fold) and Zn (by 40%). In contrast, the MT-null mice had a linear decrease in plasma Zn (-15% at 8 h, -41% at 24 h) and early loss of Zn from the liver. The Zn changes seen in MT-null mice were largely attributable to LPS-associated anorexia. Food deprivation (20 h) alone caused respective 14% and 30% decreases in hepatic and plasma Zn concentrations and a 27% reduction in total liver Zn reserves, whereas fasted normal mice conserved Zn with a 4-fold increase in hepatic MT. This study confirms that MT synthesis is essential for endotoxin-induced liver Zn accumulation.

Piccinni, E., et al. (1999). "Cadmium metallothionein gene of Tetrahymena pyriformis." Gene 234(1): 51-59.

A genomic sequence from Tetrahymena pyriformis, encoding a cadmium-induced metallothionein has been cloned. The gene encodes a transcript of 487 bases, with an intronless coding region of 324 nt, using TGA as the stop codon, TAA coding for glutamine, and the translational initiation sequence AAAATGG. Two regions of internal similarity in the coding sequence support the hypothesis that the Tetrahymena protein arose by gene duplication. The sequences of untranslated regions show some similarities with nematode MT-1 and MT-2 transcripts. Sequence of 525 bases upstream of the transcription start contains a TATA box, a CAAT box reverse complement, and many short stretches partially matching the AP-1 and ACE-1 binding sites, but no characteristic sequences found in other metallothionein promoters.

Piri, H., et al. (2012). "Construction of Plasmid Insulin Gene Vector Containing Metallothionein IIA (pcDNAMTChIns) and Carbohydrate Response Element (ChoRE), and Its Expression in NIH3T3 Cell Line." Int J Endocrinol Metab 10(3): 543-547.

BACKGROUND: Type 1 diabetes mellitus is one of the metabolic diseases that cause insulin-producing pancreatic ss cells be destroyed by immune system self-reactive T cells. Recent-ly, new treatment methods have been developed including use of the stem cells, ss islet cells transplantation and gene therapy by viral and non-viral gene constructs. OBJECTIVES: The aim of this project was preparing the non-viral vector containing the glucose inducible insulin gene and using it in the NIH3T3 cell line. MATERIALS AND METHODS: Cloning was carried out by standard methods. Total RNA was extracted from pancreatic tissue, RNA was converted to cDNA using RT-PCR reaction and preproinsulin gene was amplified using specific primers. PNMTCH plasmid was extract-ed and digested by NotI, HindIII, and MTIIA and ChoRE genes were purified and cloned into pcDNA3.1 (-) plasmid and named pcDNAMTCh. Finally, the preproinsulin genes were cloned into pcDNA3.1 (-) plasmid and pcDNAMTChIns was built. RESULTS: The cloned gene constructs were evaluated by restriction enzyme digestion and RT-PCR. The NIH3T3 cells were transfected by plasmid naked DNA containing preproinsu-lin gene and expression was confirmed by Reverse Transcriptase PCR and Western Blot-ting Techniques. CONCLUSIONS: Gel electrophoresis of PCR products confirmed that cloning was per-formed correctly. The expression of preproinsulin gene in recombinant plasmid in NI-H3T3 cell line was observed for the first time. The findings in this study can be the basis of further research on diabetes mellitus type 1 gene therapy on animals.

Plisov, S., et al. (1990). "[Identification of the glucocorticoid receptor binding site at the 5'-flanking region of mouse metallothionein I gene: the effect of base substitutions on binding efficiency]." Mol Biol (Mosk) 24(4): 1109-1116.

Interaction of highly purified glucocorticoid receptor complex (GIRC) with synthetic DNA-fragment of mouse metallotionein 1 gene promoter from -209 to -252 b.p. (MTwt) was investigated. By means of nitrocellulose filter binding assay this fragment was shown to contain specific GIRC-binding site. In order to analyse the fine structure of the site, two variants of this DNA-fragment were synthesized and used in gel retardation assay. GIRC specific binding was shown to retain throughout interaction with the fragment in which all base pairs in the surroundings of generally accepted GIRC-binding site consensus G--ACA---TGTTCT C--TGT---ACAAGA

were substituted by means of transitions, but it was weaker than the GIRC-binding with MTwt, where the mentioned consensus was situated in the natural surroundings. Complete loss of the GIRC-binding ability was observed when five CG pairs were substituted by AT ones. Two of the CG pairs belonged to the mentioned consensus. Comparison of the data obtained with results of computer analysis allows to consider the consensus as a "core" of GIRC-binding site, flanked with additional elements, interacting with GIRC.

Plisov, S., et al. (1994). "[Detection of a short segment of DNA, responsible for glucocorticoid regulation, in the 5'-flanking region on the murine metallothionein I gene]." Mol Biol (Mosk) 28(2): 407-412.

Glucocorticoid responsive element (GRE) located -252 to -209 bp upstream of the transcription initiation site of mouse metallothionein I gene (mMT I) was identified in transient gene transfer experiments. This GRE, on its own, was capable of enhancing a reporter CAT gene expression in response to dexamethasone in orientation-independent manner when linked cis to a heterologous HSV TK gene promoter. The duplication of the GRE showed a synergistic effect on dexamethasone induction of the CAT gene. Nevertheless, the GRE located in its natural promoter region of mMT I gene (-330 to +70 bp) was found to provide low if any glucocorticoid induction of linked CAT gene, while showing strong cadmium regulation, comparable to the in vivo level.

Ren, F., et al. (2011). "Cloning, characterization, expression, and copper sensitivity of the metallothionein-1 gene in the Chinese mitten crab, Eriocheir sinensis." Mol Biol Rep 38(4): 2383-2393.

A full-length metallothionein-1(MT-1) cDNA was cloned from the Chinese mitten crab, Eriocheir sinensis, based upon the hepatopancreas cDNA library. The full-length cDNA contained a single 180 bp open reading frame that encoded a 59 amino acid protein. The deduced amino acid sequence was cysteine (Cys)-rich, with residues observed in patterns characteristic of other reported MTs: Cys-X-Cys, Cys-X-X-Cys, or Cys-X-X-X-Cys. Gene structure obtained via PCR yielded a 3816 bp gene, which was comprised of three exons and two introns arranged in a "3 + 2" pattern. The cloned 5'flanking region (1,735 bp) contained several predicted binding sites, which included MREs, AP-1, SP1, USF, GATA, HNF-1, and HSF. MT-1 mRNA expression analysis revealed that while levels were highest in the hepatopancreas, expression was abundant in testis and thoracic ganglia, moderate in intestine ($P<0.05$), and weak in other tissues ($P<0.05$). MT-1 mRNA expression exhibited reproductive variation in the male, with levels approximately tenfold greater in August, during seasonal gonadal maturation, compared to other times of the year. Cu2+ exposure via tank water (0-1 mg/l for 7 days) resulted in a dose-dependent bell curve response in MT-1 mRNA expression, with peak expression observed after exposure to 0.1 mg/l Cu2+. A time course experiment (0.1 mg/l Cu2+ over 9 days) revealed MT-1 mRNA expression peaked sharply on day 5 before gradually decreasing with prolonged exposure. In the present report, we provide sequence analysis of the first MT-1 gene cloned in E. sinensis, and evidence that its physiological and toxicological regulation is evolutionary conserved.

Ren, H., et al. (2006). "Cloning of crucian carp (Carassius cuvieri) metallothionein-II gene and characterization of its gene promoter region." Biochem Biophys Res Commun 342(4): 1297-1304.

The genomic DNA of crucian carp (Carassius cuvieri) metallothionein-II (ccMT-II), with its upstream region, was obtained. The sequence analysis of its upstream region revealed several putative cis-acting elements including seven metal regulatory elements (MREs), three activator protein 1 (AP1), two glucocorticoid response elements (GREs), etc. The seven MREs locate into two clusters, a distal cluster with four MREs within -800/-600bp from the translation start site and a proximal cluster with three MREs close to TATA box. In transient luciferase gene expression assays, both of the distal and proximal cluster MREs have significantly shown synergistic effects in the transcription of ccMT-II gene; the proximal cluster of MREs serves as the major elements in metal inducing activity; Zn(2+) and Cd(2+) served as much stronger inducers than Cu(2+) shown in ccMT-II expression. The two GRE homologous sequences in ccMT-II promoter showed not to be inductive in either HepG2 or HEK293.

Ren, L., et al. (1998). "Expression of the mouse metallothionein-I gene conferring cadmium resistance in a transgenic cyanobacterium." FEMS Microbiol Lett 158(1): 127-132.

This paper reports the construction of a transgenic strain of cyanobacterium aimed at removing heavy metal pollution in waters. The mouse metallothionein-I (mMT-I) gene was inserted in the vector pRL-439 downstream of the strong psbA promoter. The resulting plasmid pRL-MT was ligated at the EcoRI site of the shuttle vector pKT-210 to generate the shuttle expression vector pKT-MT. This recombinant plasmid was introduced into Anabaena sp. PCC 7120 by triparental conjugative transfer. After selection on streptomycin, a stable transgenic Anabaena strain was obtained. The presence of the mMT-I gene was confirmed by DNA/DNA hybridization and its expression was demonstrated by immunodetection with specific antibodies. A metal tolerance experiment showed that this transgenic Anabaena strain had acquired higher metal resistance.

Ren, X. Y., et al. (2003). "Expression of metallothionein gene at different time in testicular interstitial cells and liver of rats treated with cadmium." World J Gastroenterol 9(7): 1554-1558.

AIM: Rodent testes are generally more susceptible to cadmium (Cd)-induced toxicity than liver. To clarify the molecular mechanism of Cd-induced toxicity in testes, we compared metallothionein (MT) gene expression, MT protein accumulation, and Cd retention at different time in freshly isolated testicular interstitial cells and liver of rats treated with Cd. METHODS: Adult male Sprague-Dawley rats weighing 250-280 g received a s.c injection of 4.0 micromol Cd/kg and were euthanized by CO(2) asphyxiation 1 h, 3 h, 6 h, or 24 h later. Tissue was sampled and testicular interstitial cells were isolated. There were three replicates per treatment and 3 animals per replicate for RNA analyses, others, three replicates per treatment and one animal per replicate. MT1 and MT2 mRNA levels were determined by semi-quantitative RT-PCR analysis followed by densitometry scanning, and MT was estimated by the enzyme-linked immunosorbent assay (ELISA) method. Cadmium content was determined by atomic absorption spectrophotometry. The same parametersd were also analyzed in the liver, since this tissue unquestionably accumulate MT. RESULTS: The rat testis expressed MT1 and MT2, the major isoforms. We also found that untreated animals contained relatively high basal levels of both isoform mRNA, which were increased after Cd treatment in liver and peaked at 3 h, followed by a decline. In contrast, the mRNA levels in interstitial cells peaked at 6 h. Interestingly, the induction of MT1 mRNA was lower than MT2 mRNA in liver of rat treated with Cd, but it was opposite to interstitial cells. Cd exposure substantially increased hepatic MT (3.9-fold increase), but did not increase MT translation in interstitial cells. CONCLUSION: Cd-induced expression of MT isoforms is not only tissue dependent but also time-dependent. The inability to induce the metal-detoxicating MT-protein in response to Cd, may account for a higher susceptibility of testes to Cd toxicity and carcinogenesis compared to liver.

Ren, X. Y., et al. (2003). "Metallothionein gene expression under different time in testicular Sertoli and spermatogenic cells of rats treated with cadmium." Reprod Toxicol 17(2): 219-227.

The rodent testes are generally more susceptible to cadmium (Cd)-induced toxicity than the liver. To clarify the molecular mechanism underlying tissue and cell differences in Cd sensitivity, we compared metallothionein (MT) gene expression, MT protein accumulation, and Cd retention under different times in freshly isolated testicular Sertoli and spermatogenic cells and liver of rats treated with Cd. Adult male Sprague-Dawley rats received a s.c. injection of 4.0 micromol Cd/kg and 1, 3, 6, or 24h later and untreated animals (0h) tissue were sampled and testicular Sertoli and spermatogenic cells isolated. MT1 and MT2 mRNA levels were determined by semi-quantitative RT-PCR analysis followed by densitometry scanning, and MT was estimated by the enzyme-linked immunosorbent assay (ELISA) method. Cadmium content was determined by atomic absorption spectrophotometry. Testicular lesions were not grossly or histologically observed in rats treated with 4.0 micromol Cd/kg. In the present study, we demonstrated that the rat testis indeed expressed MT1 and MT2, the major isoforms. We also found that untreated animals contained relatively high basal levels of both isoform mRNA, which were increased after Cd treatment in liver and peaked at 3h, followed by a decline, in contrast, the mRNA levels in Sertoli cells peaked at 6h. Interestingly, the induction of MT1 mRNA was lower than MT2 mRNA in Sertoli cells and liver of rats treated with Cd. However, the MT1 mRNA levels of spermatogenic cells decreased 0-3h after Cd treatment, followed by an increase; in contrast, MT2 mRNA levels increased 0-3h after Cd treatment, followed by a reduction, but induced extents of them are lower than those of Sertoli cells and liver. Cd exposure substantially increased hepatic MT, but did not increase MT translation in Sertoli and spermatogenic cells. These results indicate: (1) that Cd-induced MT mRNA expression is cell- and time-dependent; (2) that the inability to induce the metal-detoxicating MT protein in response to Cd, might account for higher susceptibility of testes to Cd toxicity and carcinogenesis relative to liver.

Ren, Y. and A. Smith (1995). "Mechanism of metallothionein gene regulation by heme-hemopexin. Roles of protein kinase C, reactive oxygen species, and cis-acting elements." J Biol Chem 270(41): 23988-23995.

Heme-hemopexin or cobalt protoporphyrin (CoPP)-hemopexin (a model ligand for hemopexin receptor occupancy) is shown to increase transcription of the metallothionein-1 (MT-1) gene by activation of a signaling pathway. Promoter deletion analysis followed by transient transfection assays show that 110 base pairs (-153 to -43) of 5'-flanking region of the murine MT-1 promoter are sufficient for increasing transcription in response to heme-hemopexin or to CoPP-hemopexin in mouse hepatoma cells. The protein kinase C inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7), prevented the increase in MT-1 transcription by heme-hemopexin, CoPP-hemopexin, or phorbol 12-myristate 13-acetate, but the protein kinase A inhibitor, HA1004, was without effect. N-Acetylcysteine (NAC) and glutathione, as well as superoxide dismutase and catalase, inhibited both the increase in endogenous MT-

1 mRNA and the activation of reporter gene activity by heme-hemopexin, CoPP-hemopexin, and phorbol 12-myristate 13-acetate. In sum, these data suggest that reactive oxygen intermediates are generated by heme-hemopexin via events associated with receptor binding, including protein kinase C activation. Induction of heme oxygenase-1 expression, in contrast to MT-1, is significantly less sensitive to NAC. Deletion and mutation analyses of the MT-1 proximal promoter revealed that the sequence 5'-GTGACTATGC-3' (from -98 to -89 base pairs) is, in part, responsible for the hemopexin-mediated regulation of MT-1 which is inhibited by H7. Regulation via this element is also induced by H2O2 showing that it is an antioxidant response element. Heme itself acts via more distal elements on the MT-1 promoter. In contrast to NAC and glutathione, diethyl dithiocarbamate and pyrrolidine dithiocarbamate, which inactivate reactive oxygen intermediates and chelate Zn(II), synergistically augment the induction of MT-1 mRNA levels and reporter gene activity in response to heme-hemopexin via the antioxidant response element by both metal-responsive element-dependent and -independent mechanisms.

Ren, Y. and J. Zhao (2009). "Functional analysis of the rice metallothionein gene OsMT2b promoter in transgenic Arabidopsis plants and rice germinated embryos." Plant Sci 176(4): 528-538.

In this paper, the promoter of a type 2 metallothionein gene OsMT2b was cloned in indica rice Jiayu948 and its function was analyzed in transgenic Arabidopsis plant and rice germinated embryo aided by a GUS reporter gene. The result shows that the full promoter drives GUS expression predominantly in the vascular tissues of Arabidopsis, and the expression undergoes a unimodal pattern during the development, with peaking in the mature tissues in leaves and floral organs. Further promoter deletion analysis in Arabidopsis displays different function regions that are crucial for regulating gene expression: the -212/-21 region for keeping the minimal promoter activity and the expression in the initiation site of lateral root; the -924/-213 region for the expression in vegetative and reproductive organs; the -1227/-925 region for confining high expression in silique; and the -1502/-1228 and -1227/-925 regions for the balanceable control of high expression in embryo. And by using a transient expression system in rice germinated embryo, the similar promoter region-based regulation was observed. In addition, from studying the promoter activities under different stress conditions such as ABA, GA, ZT, PEG, cold, hot, NaCl, Cu, Zn and wounding, it is proposed that environmental stresses may regulate OsMT2b expression through the promoter cis-acing elements.

Reynolds, T. L. and R. L. Crawford (1997). "Effects of light on the accumulation of abscisic acid and expression of an early cysteine-labeled metallothionein gene in microspores ofTriticum aestivum during induced embryogenic development." Plant Cell Rep 16(7): 458-463.

A cloned cDNA to the wheat (Triticum aestivum) early cysteine-labeled metallothionein has many characteristics of a molecular marker for pollen embryogenesis in this plant. This transcript was not detected in uninucleate microspores at the time of culture or in pollen at any stage during normal ontogeny; its mRNA did begin to increase in embryogenic microspores within 6 h of culture, peaked at around 24 h, declined, then leveled off through the 21-day-old embryoid stage. Additionally, the accumulation of the embryoid-abundant EcMt gene transcript showed a direct and positive correlation with an increase of ABA in embryogenic microspores and developing pollen embryoids. Irradiating cultures with high intensity white light or with far-red, or blue light, suppressed EcMt transcript accumulation and the ability of microspores to form embryoids; however, light did not affect ABA concentrations during the early stages of culture. These results suggest that although a promoter of pollen embryogenesis in bread wheat, ABA alone cannot maintain the sporophytic differentiation of microspores subjected to inhibitory regimes of light in vitro. Whether or not light acts directly or indirectly in suppressing EcMt gene expression and pollen embryogenesis remains unknown.

Reynolds, V. L., et al. (1987). "Regulation of a metallothionein-rasT24 fusion gene by zinc results in graded alterations in cell morphology and growth." Oncogene 1(3): 323-330.

We constructed fusion genes consisting of the mouse metallothionein I (MT) 5' region and the coding region of either the human H-ras gene (c-rasP3) or a mutated allele (c-rasT24); both ras genes lacked the initial (non-coding) exon and the first 30 bp of the non-coding region of the second exon. Transfection of Rat-1 cells produced foci only with pMT-rasT24, and selection in soft agar yielded clones in which MT-rasT24 expression was zinc-regulatable. In response to increasing concentrations of ZnSO4, these lines showed increasingly altered morphology (conversion to fusiform or spheroidal morphology), progressively higher maximal cell density, and an increasingly greater fraction of cells in the S + G2 + M portion of the cell cycle at high density. MT-rasT24 RNA levels in zinc-responsive lines were increased between 4- and 6-fold by the addition of ZnSO4 (final concentration = 100 microM) to the medium. Replating cells in the absence of zinc reversed the biological effects and resulted in reduction in MT-rasT24 RNA levels. Thus, graded alterations in phenotype result from increasing

levels of MT-rasT24 gene expression.
Rhee, J. S., et al. (2009). "Differential expression of metallothionein (MT) gene by trace metals and endocrine-disrupting chemicals in the hermaphroditic mangrove killifish, Kryptolebias marmoratus." Ecotoxicol Environ Saf 72(1): 206-212.

Metallothionein (MT) gene expression was studied in different tissues, development stages and gender types of the mangrove killifish (Kryptolebias marmoratus). MT expression was also studied in a time-series experiment after exposure to trace metals and endocrine-disrupting chemicals (EDCs). The brain showed the highest level of MT transcripts. Although all the development stage showed some level of MT expression, the adult hermaphrodites showed the highest expression which was significantly higher than the secondary males. In the trace metal-exposed fish, cadmium caused the strongest induction of MT. However, other trace metals such as copper and zinc also caused MT gene induction. All the EDCs suppressed the expression of MT gene, and the effect of EDCs were not gender-specific. K. marmoratus has previously shown its suitability as a model species for toxicity studies and cancer research. This study demonstrated utility of MT as biomarker in K. marmoratus. However, confounding factors such as age, gender, and tissue types appear to influence the MT expression. Response of trace and organic pollutants such as EDCs also varied greatly. These observations suggest that MT would be a specific biomarker of trace metal exposure in K. marmoratus and expression would be influenced by intrinsic factors.
Richards, R. I., et al. (1984). "Structural and functional analysis of the human metallothionein-IA gene: differential induction by metal ions and glucocorticoids." Cell 37(1): 263-272.

We describe a region of human DNA containing four metallothionein (hMT) genes. One of these genes, hMT-IA, was found to encode a functional protein that confers heavy metal resistance to NIH 3T3 cells after transfer on a bovine papilloma virus-derived vector. This gene is expressed in cultured human cell lines, but at a lower basal level than the hMT-IIA gene; it shows a different induction response to heavy metals and glucocorticoids than the hMT-IIA gene. Induction of the human MT family therefore does not represent an equivalent elevation in the level of expression of individual genes, but is the sum of the differential responses of active members. The differential response is due to functional differences of the respective promoter/regulatory regions of the genes as shown by gene-fusion experiments. While the hMT-IIA promoter is responsive to Cd++, Zn++, and glucocorticoids, the hMT-IA promoter mediates response only to Cd++.
Sacky, J., et al. (2019). "Different cadmium tolerance of two isolates of Hebeloma mesophaeum showing different basal expression levels of metallothionein (HmMT3) gene." Fungal Biol 123(3): 247-254.

Hebeloma mesophaeum is an ectomycorrhizal fungus frequently associated with metal disturbed environments. In this work, we examined Ag, Cd, and Zn tolerance of H. mesophaeum isolates from heavy metal-polluted (isolate Prib) and clean (isolate Rez) sites. Both mycelia showed essentially the same level of Ag and Zn tolerance, but Prib was more Cd tolerant. In short-term exposures, Prib accumulated slightly less Cd than Rez. Size exclusion chromatography of cell-free extracts and fluorescence microscopy of hyphae with a Cd-specific fluorescent tracer revealed that substantial proportion of Cd was contained in the vacuoles in both isolates. Considering that the proportion of Cd associated with fractions attributable to Cd complexes with cytosolic, metallothionein (MT) peptides was higher in Prib, we examined the copy number and basal levels of HmMTs genes in Rez and Prib. While no difference between the isolates was observed in the gene copy numbers and basal levels of HmMT1 transcripts, the basal transcription of HmMT3 was 3-fold higher in Prib. These observations suggest that MTs provide in Prib better protection against Cd. Furthermore, the higher Cd tolerance in Prib can be to some extent also supported by the efflux or reduced uptake of Cd in the hyphae.
Saeed, O. A., et al. (2019). "Effects of corn supplementation on the antioxidant activity, selected minerals, and gene expression of selenoprotein and metallothionein in serum, liver, and kidney of sheep-fed palm kernel cake: urea-treated rice straw diets." 3 Biotech 9(4): 146.

This study aimed to determine influence of corn inclusion on glutathion peroxidase (GPx) activity, selected minerals concentration, and gene expression in sheep-fed palm kernel cake (PKC) and urea-treated rice straw. Twenty-seven of Dorper sheep were divided into three groups and fed a basal diet of (20% rice straw and 80% concentrate) with addition of ground corn at either 0% (T1), 5% (T2), or 10% (T3), respectively. After 120 days feeding trial, all animals were slaughtered and tissue samples of kidney, liver, and muscles were taken for enzyme and mineral analyses. The results showed that Cu concentration in the liver was lower treatment T3 compared to the control and T2. The serum activity of GPx was higher in T2 than in T3 at day 120 of experiment. Serum malondialdehyde (MDA) concentrations decreased at day 80 in sheep on T3, whereas MDA of liver increased linearly with increasing corn supplementation. The qRT-PCR analyses revealed significant up-regulation of ATP7A and MIa genes in T3, while hepatic Cu/Zn SOD, GPx1, and GPx4 mRNA showed a higher expression in lamb hepatocytes in T3 compared to those on T1. Present study results suggest that feeding PKC as basal diet can

increase antioxidant activity, but cause liver dysfunction in sheep. Inclusion corn was found to regulate transcriptional levels of the GPx family and metallothionein genes. These genes may play a role in the antioxidant protection response and reduce incidence of toxicity associated with Cu.

Saijoh, K., et al. (1994). "Brain metallothionein gene expression and regulation." Biol Signals 3(3): 150-156.

Metallothionein (MT) gene expression in the brain has been most thoroughly studied using rodents. Although MT is considered to be a 'housekeeping' protein even in the brain, the basal MT mRNA expression level is not always high. Differences in the responses of rats and mice have made it difficult to interpret the data. Moreover, the response to inducers is not always apparent, probably because the brain is protected by the blood-brain barrier and initial responses to inducers in peripheral tissues modulate their accumulation in the brain. A relatively high content of MT protein in the brain might be sufficient to elicit minute alterations in the level of inducers. Nonetheless, regulation of MT gene expression in the brain seems to be important in e.g. maintaining the levels of trace elements and controlling redox potentials. The localization and utilization of trans elements such as MTF-I and MEP-I in the brain will provide new aspects for study. The high homology among MT isoforms with respect to nucleotide as well as amino acid sequences has made it difficult to obtain cDNA probes or antibodies capable of distinguishing MT isoforms. Thus, their cross-reactivity might make changes in MT mRNAs appear minimal when MT isoforms are differently regulated. The rapid developments in methodology permitting sensitive, rapid, high-resolution analysis could clarify the background of tissue- and cell-specific gene regulation as well as differential induction.

Saijoh, K., et al. (1989). "Molecular cloning of cDNA for rat brain metallothionein-II and regulation of its gene expression." Pharmacol Toxicol 64(5): 464-468.

A rat brain metallothionein-II (MT-II) complementary DNA (cDNA) clone was isolated from a cDNA plasmid library, which was prepared from non-treated rat brain mRNA, by a colony screening procedure using 32P-labeled synthetic oligonucleotide probes. It is deduced that the clone encodes for a protein of 61 amino acids comprising 20 cysteines, which is highly homologous to MT-IIs in other species. Northern blot analysis demonstrated major mRNA species in the brain, liver and kidneys (approximately 350 b in size), which is induced in response to dexamethasone, zinc, cadmium and mercury but not to methyl mercury. These findings confirm that MT-II genes are expressed and regulated both by steroid and heavy metals in the brain as well as in peripheral organs.

Saint-Jacques, E., et al. (1995). "Structure and metal-regulated expression of the gene encoding Xenopus laevis metallothionein-A." Gene 160(2): 201-206.

To investigate the regulation of amphibian metallothionein(MT)-encoding genes, we have isolated and sequenced the XlMT-A gene encoding Xenopus laevis (Xl) MT-A. The gene displays an overall structure similar to that of mammalian MT, with three exons interrupted by two introns. The promoter region contains a typical TATA box and two metal regulatory elements (MRE) within the first 100 bp upstream from the transcription start point (tsp). The transition metal ion (Mc2+) inducibility of the promoter was studied by transient expression experiments in CV-1 African green monkey kidney cells, using different DNA fragments from the 5'-flanking region of XlMT-A fused to the bacterial cat reporter gene. The first 145 bp upstream from the tsp are sufficient to confer inducibility of cat by Cd2+. Constructs bearing only the most proximal MRE are not inducible by Me2+, thus suggesting that both MRE are required for Me2+ induction. Recognition sites for the transcription factors, AP-1 and AP-2, are located within the first 180 bp of the promoter region and these elements appear to be involved in controlling the constitutive basal level of expression of this MT.

Saint-Jacques, E., et al. (1998). "Cloning of a complementary DNA encoding an Ambystoma mexicanum metallothionein, AmMT, and expression of the gene during early development." DNA Cell Biol 17(1): 83-91.

We have used a polymerase chain reaction strategy to isolate a metallothionein (MT) cDNA from the amphibian Ambystoma mexicanum (axolotl). This cDNA is 875-bp long and encodes a 60 amino acid protein, AmMT, typical for family 1 MTs. It contains 20 cysteine (Cys) residues that can be aligned with those of other vertebrate MTs. The overall structure of the protein is unique among vertebrates in having only two amino acid residues before the first Cys at the amino-terminal end. Northern analyses showed that AmMT is expressed throughout embryogenesis, giving rise to three mRNA species of 650, 750, and 1,600 nucleotides (nt). The 750 and 1,600 nt transcripts appear to result from differential use of polyadenylation signals, whereas the 650 nt RNA could arise from deadenylation of the 750-nt transcript. Both the 750- and 1,600-nt RNAs were presented in embryos before the mid-blastula transition (MBT). After the MBT, the 750-nt RNA was replaced by the 650-nt RNA which was gradually degraded to undetectable levels in post-neurulation embryos. Levels of the 1,600-nt transcript increased at gastrulation and reach a maximum in Stage 30 embryos. In adult animals, levels of the 750-nt RNA were high in liver and testes, and very low in lung, gut, skin, and oviducts,

whereas levels of the 1,600-nt transcript were similar and moderately elevated in all tissues examined. In contrast, in Xenopus laevis, Northern analysis did not detect XIMT-A mRNA in embryos before late neurulation (Stage 24). XIMT-A mRNA levels then increased sharply in Stage 36 hatched embryos at levels similar to those found in adult livers. These results show that AmMT presents a unique expression pattern among metazoans being transcribed as two transcripts differing in the length of their 3' untranslated regions, the levels of which vary during embryogenesis and in adult tissues.

Sakatoku, A., et al. (2020). "Molecular Identification, Characterization, and Expression Analysis of a Metallothionein Gene from Septifer virgatus." Mar Biotechnol (NY) 22(4): 488-497.

This study provides a preliminary characterization of a metallothionein (MT) gene in Septifer virgatus and highlights its potential use in biomonitoring. The full-length SvMT cDNA and the complete sequence of the SvMT gene were identified using reverse transcriptase PCR coupled with the rapid amplification of cDNA ends and the primer walking method. The SvMT cDNA encodes a protein of 72 amino acids having nine classical Cys-X-Cys motifs. Moreover, the deduced amino acids contained the conserved motif (Cys-x-Cys-x(3)-Cys-Thr-Gly-x(3)-Cys-x-Cys-x(3)-Cys-x-Cys-Lys) of MT family 2. Its molecular mass and isoelectric point were estimated to be 7.01 kDa and 7.00, respectively. BLAST-based searching indicated that SvMT shared 81.0% amino acid sequence identity with Mytilus edulis MT-20-II. The SvMT gene has three coding exons and two introns. After exposure to 1 mg/L cadmium chloride, the expression of SvMT increased 15-fold by 3 days (d), with a maximum expression of 27-fold by 5 d compared with the pre-exposure level. After exposure to 2 mg/L zinc chloride, the expression of SvMT increased 2.5-fold by 3 d and 4.7-fold by 5 d compared with the pre-exposure level. A significant increase in the expression level of SvMT mRNA was observed after the exposure of S. virgatus to the combination of 0.003 mg/L cadmium chloride and 0.2 mg/L zinc chloride compared with the pre-exposure level. Our work indicates that the SvMT gene is associated with stress responses and could be a potential biomarker for marine pollution.

Samardzic, J. T., et al. (2010). "Tissue expression analysis of FeMT3, a drought and oxidative stress related metallothionein gene from buckwheat (Fagopyrum esculentum)." J Plant Physiol 167(16): 1407-1411.

Metallothionein type 3 (MT3) expression has previously been detected in leaves, fruits, and developing somatic embryos in different plant species. However, specific tissular and cellular localization of MT3 transcripts have remained unidentified. In this study, in situ RNA-RNA analysis revealed buckwheat metallothionein type 3 (FeMT3) transcript localization in vascular elements, mesophyll and guard cells of leaves, vascular tissue of roots and throughout the whole embryo. Changes in FeMT3 mRNA levels in response to drought and oxidative stress, as well as ROS scavenging abilities of the FeMT3 protein in yeast were also detected, indicating possible involvement of FeMT3 in stress defense and ROS related cellular processes.

Samson, S. L. and L. Gedamu (1995). "Metal-responsive elements of the rainbow trout metallothionein-B gene function for basal and metal-induced activity." J Biol Chem 270(12): 6864-6871.

In this study, the contributions of the two metal-responsive elements (MREs) of the rainbow trout (Salmo gairdnerii) metallothionein (tMT)-B gene promoter (-137 to +5) were analyzed. The effect of MRE mutations on the basal and zinc-induced activities of tMT-B promoter-reporter gene fusions were determined by transfection of a rainbow trout hepatoma (RTH-149) cell line. Together, MREa and MREb cooperate to elicit a significant response to zinc but exhibit differential basal and metal-induced activity. The MREa sequence (-62 to -51) is important for basal promoter activity and can function independently, whereas the more distal MREb (-89 to -100) mainly contributes to metal induction through cooperative interactions with MREa. The degree of basal character of the MREs is partially determined by nucleotide differences at the flexible position N of the MRE consensus TGC(G/A)CNC. In mouse L and HepG2 cells, MREa activity is conserved, but the contributions of the MREb region differ, including reduced cooperativity with MREa. There are also differences in the apparent molecular masses of the rainbow trout and mammalian nuclear factors that bind to the tMT-B promoter and MREa sequence.

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

Metallothionein (MT) genes encode small proteins that chelate metal ions through metal-thiolate bonds with cysteine residues. MTs may have a role in cellular zinc homeostasis and metal detoxification. Congruent with these putative functions, MT gene transcription is induced by metals via multiple metal-responsive elements (MREs) present in the MT gene 5'-regulatory regions. This chapter mainly is focused on studies of the functional and physical interactions of MRE binding proteins with MT promoters from human and rainbow trout. In addition to mediating zinc induction, MREs may make important contributions to nonmetal induced promoter activity. In part, differential basal activity of MREs appears to be determined by

sequence and position in the promoter. During zinc induction, increased functional MRE activity correlates with increased activity of mammalian MRE binding proteins by zinc treatment in vivo or in vitro, as detected by electrophoretic mobility shift assays. Interestingly, the addition of cadmium in vitro or in vivo has no detectable effect even though it strongly induces MT gene expression in the same time course. This raises questions about how the effects of cadmium are mediated by MREs. The molecular masses and MRE complex migration of the zinc-responsive factors we detect are consistent with mouse and human metal-responsive transcription factor (MTF) and expression of the MTF cDNAs increases co-transfected MT promoter activity in both mammalian and trout cell lines underlining the conservation of MRE binding factor function among diverse species.

Samson, S. L., et al. (2001). "The rainbow trout metallothionein-B gene promoter: contributions of distal promoter elements to metal and oxidant regulation." Biochim Biophys Acta 1517(2): 202-211.

In this report, the contributions of the distal 5'-regulatory sequences of the rainbow trout (Oncorhynchus mykiss) metallothionein (tMT)-B gene promoter (-738 to +5) were studied. Transfection of the -738 promoter fragment in a rainbow trout hepatoma cell line (RTH-149) resulted in 4- to 5-fold greater activity compared to the proximal -137 promoter region. Mutation of the proximal MREa abolishes the basal activity of the -738 fragment indicating that the distal regulatory elements require a cooperative interaction with MREa. However, the fragments containing both distal MREs, c and d (positioning -570 and -680, respectively), or MREc alone could confer basal and metal-induced activity when fused to the TATA box. This suggests that these distal elements are functional and therefore may play a role as basal elements in their natural state. The trout MT genes are also induced by oxidants including H2O2, tBHP and tBHQ. The larger promoter fragment -738 responds to H2O2, while the -137 fragment does not. However, fusion of the isolated MREc fragment (-648 to -533) in its native orientation, upstream of the -137 promoter elicits a response to H2O2, although no response is seen with MREc in reverse. These data suggest that this distal fragment contains functional oxidant responsive elements which have resemblance to the mammalian antioxidant responsive element (AREs).

Samson, S. L., et al. (1995). "Functional analyses of the human metallothionein-IG gene. In vitro and in vivo studies." J Biol Chem 270(42): 25194-25199.

We have analyzed the human (h) metallothionein (MT)-IG proximal promoter region (-174 to +5) using a TATA box mutation (TATCA) and four trinucleotide mutants of the proximal MREa. Transient transfection of HepG2 cells was complemented by in vitro transcription with rat liver nuclear extracts. In both systems, mutations of the TATA box and conserved core of metal responsive element (MRE)a were detrimental to hMT-IG promoter activity suggesting that both elements make significant contributions to hMT-IG transcription. Although MRE binding factors were active in vitro, further metal activation of MT promoter activity was accomplished only by in vivo metal treatment rather than addition of zinc in vitro. Southwestern blotting identified nuclear proteins in rat liver and HepG2 cells which physically interact with MREa in a zinc-dependent manner and could be responsible for MREa function in each system. In addition, the functional effects of the TATCA mutation correlate with altered physical interaction with TATA box-binding protein as observed using DNase I protection.

Santon, A., et al. (2003). "Metallothionein-1 and metallothionein-2 gene expression and localisation of apoptotic cells in Zn-treated LEC rat liver." Histochem Cell Biol 119(4): 301-308.

The aims of the present work were to determine the effect of long-term treatment with zinc (Zn) on metallothionein (MT) concentrations and to study the levels of both MT-1 and MT-2 mRNAs in Long-Evans Cinnamon (LEC) rat liver. We also identified apoptotic cells comparing two cytochemical techniques. Thirteen rats received 50 mg zinc acetate daily by gavage, 13 rats received no treatment, and both groups were killed after 60 days. Finally four rats were killed 35 days after birth (T(0)). The results demonstrate that the Zn-treated group had higher levels of MT than both the untreated and basal ones. Quantification of mRNA indicates that the level of the Zn-treated group was significantly higher than the untreated group. Confocal fluorescent staining with monoclonal antibody (Mab) against single-strand DNA localised the hepatic cells that had chromatin condensation and nuclear fragmentation typical of apoptosis, especially in the untreated group sections. The intensity and quantity of fluorescence decreased in both the treated and basal groups. The higher sensitivity of Mab staining compared to TUNEL, which revealed both apoptotic and necrotic cells, reflects the different action mechanism of the two techniques. These findings confirm, in LEC rats, the important role of Zn in cellular protection in relation to MT expression and apoptotic processes as cellular responses to DNA damage by free radicals.

Sarkar, S., et al. (2004). "Effects of heavy metals on population growth and metallothionein gene expression in the mosquito Culex quinquefasciatus, from Calcutta, India." Environ Pollut 127(2): 183-193.

Major water bodies in and around the city of Calcutta (India) receive heavy metal contaminated effluents from industries, households, and vehicular

traffic through sewage or drainage. We quantified concentrations of Cu, Zn, and Cd from three water bodies at Kalighat, Tangra, and VIP Road, respectively. The concentrations of these heavy metals were significantly greater in the summer than in monsoon when heavy downpours resulted in reduced metal concentrations. Concentrations of metals in the mosquito Culex quinquefasciatus also reflected such seasonal fluctuations. Hatchability and survivorship of C. quinquefasciatus significantly differed among the sites and were reduced significantly from the control. Exposure to heavy metals also induced MT-gene expression in C. quinquefasciatus, likely helping them to survive in the water bodies stressed with heavy metals. MT-gene activity demonstrated significant variation among sites and seasons with the highest activity in the summer in the VIP Road population. This study suggests that C. quinquefasciatus could be used as an ecological indicator of heavy metal pollution by monitoring its MT-gene expression.

Sasaki, H., et al. (1989). "Activity of a metallothionein-transthyretin fusion gene in transgenic mice. Possible effect of plasmid sequences on tissue-specific expression." Mol Biol Med 6(4): 345-353.

Three strains of transgenic mice carrying the mouse metallothionein-I (MT) promoter fused to the human transthyretin (TTR) structural gene plus pUC plasmid sequences were investigated for expression of the fusion gene. Human TTR was inducible in the serum of at least two strains and the fusion gene mRNA was detected in several tissues of all the strains. The testis showed constitutive mRNA synthesis, while the intestine and some other tissues showed inducible expression. Unexpectedly, however, the fusion gene activity was grossly suppressed in the liver and kidney of all the strains. Available data suggest that this suppression results from the presence of the plasmid sequences. Analysis of tissue DNAs shows that the methylation status of the promoter sequences varies from strain to strain, depending on their chromosomal position, and that some CpG sites in the proximal portion of the promoter are not methylated at all in the liver and kidney of two strains. These findings suggest that the plasmid sequences suppress the MT promoter activity in some specific tissues by a mechanism that does not involve DNA methylation.

Sato, M., et al. (2000). "Analysis of the metallothionein gene in age-related macular degeneration." Jpn J Ophthalmol 44(2): 115-121.

PURPOSE: To analyze the expression of metallothionein in neovascular membranes in patients with age-related macular degeneration (AMD) and to compare the findings in patients with proliferative diabetic retinopathy and proliferative vitreoretinopathy with findings in normal retinal pigment epithelial (RPE) cells. METHODS: Semiquantitative reverse transcriptase-polymerase chain reaction and immunohistochemical examinations were performed. Sequence analysis was also carried out. RESULTS: Expression of the metallothionein-II gene was not statistically significant in proliferative membranes. Sequence analysis revealed that there were at least six polymorphisms in the metallothionein-II gene and three in the metallothionein-IA gene. However, no amino acid substitution was observed. Metallothionein expression was also observed by immunohistochemical techniques in RPE cells of neovascular membranes in AMD patients. CONCLUSIONS: Metallothionein was reported to be decreased in aged macular RPE and in some monkey retina with early onset of macular degeneration. However, our results suggest that the expression of the metallothionein-II gene in neovascular membranes in patients with AMD may be controlled like other proliferating cells.

Savva, D., et al. (2002). "Characterisation of DNA probes for the analysis of metallothionein gene expression in the bank vole (Clethrionomys glareolus)." Environ Int 28(3): 139-146.

DNA probes have been developed for subsequent use in monitoring the exposure of animals to heavy metal pollution in terrestrial environments using metallothionein (MT) gene expression in the bank vole (Clethrionomys glareolus). Three different bank vole sequences were characterised corresponding to the cDNA and the genomic DNA for MT-I and the genomic DNA for MT-II. Nucleotide sequence analysis indicates that the coding sequences of the bank vole MT-I and MT-II genes exhibit a very high degree of similarity (greater than 92%) to the corresponding genes of the Chinese hamster, the mouse and the rat. In common with other mammalian MT genes, both the MT-I and MT-II genes in the bank vole are interrupted by two introns, which are at identical positions as those in other rodent MT genes; furthermore, the sizes of these introns are similar to those in other rodents with the first intron being larger than the second and those in the MT-I gene being larger than those in the MT-II gene. The predicted amino acid sequence for the proteins shows that both proteins contain 20 cysteine residues at positions identical to those in other known mammalian MTs. The availability of these DNA sequences now provides a good opportunity to investigate MT gene expression and possible gene amplification in bank voles exposed to metal pollution.

Sayers, Z., et al. (1993). "Cloning and expression of Saccharomyces cerevisiae copper-metallothionein gene in Escherichia coli and characterization of the recombinant protein." Eur J Biochem 212(2): 521-528.

The gene sequences for intact and truncated forms of copper-binding metallothionein from Saccharomyces cerevisiae were cloned and overexpressed in Escherichia coli BL21(DE3)pLysE

cells. In contrast to several other genes, the intact and truncated metallothionein genes are amplified in the polymerase chain reaction when Mg2+ is replaced by Co2+. The recombinant truncated protein binds copper in vivo and in vitro. A ratio of 8 Cu/12 cysteines was determined from atomic absorption, X-ray fluorescence and amino acid analysis. Extended X-ray absorption spectroscopy indicates that all Cu is in Cu(I) form and coordinated to three S atoms.

Scanlon, K. J., et al. (1991). "Ribozyme-mediated cleavage of c-fos mRNA reduces gene expression of DNA synthesis enzymes and metallothionein." Proc Natl Acad Sci U S A 88(23): 10591-10595.

The c-fos gene product Fos has been implicated in many cellular processes, including signal transduction, DNA synthesis, and resistance to antineoplastic agents. A fos ribozyme (catalytic RNA) was designed to evaluate the effects of suppressing Fos protein synthesis on expression of enzymes involved in DNA synthesis, DNA repair, and drug resistance. DNA encoding the fos ribozyme (fosRb) was cloned into the pMAMneo expression plasmid, and the resultant vector was transfected into A2780DDP cells resistant to the chemotherapeutic agent cisplatin. The parental drug-sensitive A2780S cells were transfected with the pMMV vector containing the c-fos gene. Morphological alterations were accompanied by significant changes in pharmacological sensitivity in both c-fos- and fosRb-transfected cells. pMAMneo fosRb transfectants revealed decreased c-fos gene expression, concomitant with reduced thymidylate (dTMP) synthase, DNA polymerase beta, topoisomerase I, and metallothionein IIA mRNAs. In contrast, c-myc expression was elevated after fos ribozyme action. Insertion of a mutant ribozyme, mainly capable of antisense activity, into A2780DDP cells resulted in smaller reductions in c-fos gene expression and in cisplatin resistance than the active ribozyme. These studies establish a role for c-fos in drug resistance and in mediating DNA synthesis and repair processes by modulating expression of genes such as dTMP synthase, DNA polymerase beta, and topoisomerase I. These studies also suggest the utility of ribozymes in the analysis of cellular gene expression.

Seguin, C., et al. (1984). "Competition for cellular factors that activate metallothionein gene transcription." Nature 312(5996): 781-785.

Metallothioneins (MTs), small cysteine-rich proteins, bind to and are inducible by heavy metals such as zinc, cadmium and copper. Recent gene-transfer and mutagenesis experiments have elucidated cis-acting DNA sequences involved in this form of regulation, but nothing is known about the trans-acting factors that interact with the control sequences or how such interactions influence the rate of transcription. We report here the detection of cellular factors involved in the cadmium induction of the mouse MT-1 gene by an in vivo competition assay. We show that at least one-class of these cellular factors acts by a positive regulatory mechanism depending on the same region of the 5' flanking DNA required for maximal transcription.

Seguin, C. and D. H. Hamer (1987). "Regulation in vitro of metallothionein gene binding factors." Science 235(4794): 1383-1387.

Mouse nuclear factors that bind to an upstream metal regulatory element of the mouse metallothionein-I gene have been identified by DNA footprinting and oligonucleotide band shift assays. The formation of complexes at this site can be activated 20- to 40-fold by the vitro addition of ionic cadmium. The activation reaction is rapid, reversible by a metal chelator, and may involve multiple proteins. These results suggest that the initial step in cadmium detoxification is an interaction between the metal and nuclear DNA-binding factors leading to an increase in metallothionein gene transcription. The ability to observe metal activation in vitro makes this a powerful system to study the biochemistry of eukaryotic gene regulation.

Seguin, C. and J. Prevost (1988). "Detection of a nuclear protein that interacts with a metal regulatory element of the mouse metallothionein 1 gene." Nucleic Acids Res 16(22): 10547-10560.

Metallothionein (MT) genes contain multiple metal regulatory elements (MREs) that are responsible for metal induction. A protein blotting procedure and a synthetic oligonucleotide have been used to identify nuclear factors interacting with a MRE (MREd) of the mouse MT-1 gene. We report the specific binding of the probe to a protein of apparent Mr 108,000 (p108). The specificity of the interaction was demonstrated by mutation analysis and competition experiments. Furthermore, the probe contains the Sp1 consensus binding sequence 5'CCGCCC3', in addition to the MRE consensus sequence, 5'TGCAC3', and we show that a Simian Virus 40 DNA fragment which contains six Sp1 binding sites did not bind p108 nor did it compete for the protein(s) interacting with MREd in a DNA footprinting assay. These results show that a metal regulatory element of the mouse MT-1 gene interacts specifically with a nuclear protein of Mr 108,000 and that this protein is distinct from the transcription factor Sp1.

Sekovanic, A., et al. (2020). "Metallothionein 2A gene polymorphisms in relation to diseases and trace element levels in humans." Arh Hig Rada Toksikol 71(1): 27-47.

Human metallothioneins are a superfamily of low molecular weight intracellular proteins, whose synthesis can be induced by essential elements (primarily Zn and Cu), toxic elements and chemical

agents, and stress-producing conditions. Of the four known isoforms in the human body MT2 is the most common. The expression of metallothioneins is encoded by a multigene family of linked genes and can be influenced by single nucleotide polymorphisms (SNPs) in these genes. To date, 24 SNPs in the MT2A gene have been identified with the incidence of about 1 % in various population groups, and three of them were shown to affect physiological and pathophysiological processes. This review summarises current knowledge about these three SNPs in the MT2A gene and their associations with element concentrations in the body of healthy and diseased persons. The most investigated SNP is rs28366003 (MT2A -5 A/G). Reports associate it with longevity, cancer (breast, prostate, laryngeal, and in paranasal sinuses), and chronic renal disease. The second most investigated SNP, rs10636 (MT2A +838G/C), is associated with breast cancer, cardiovascular disease, and type 2 diabetes. Both are also associated with several metal/metalloid concentrations in the organism. The third SNP, rs1610216 (MT2A -209A/G), has been studied for association with type 2 diabetes, cardiomyopathy, hyperglycaemia, and Zn concentrations. Metallothionein concentrations and MT2A polymorphisms have a potential to be used as biomarkers of metal exposure and clinical markers of a number of chronic diseases. This potential needs to be studied and verified in a large number of well-defined groups of participants (several hundreds and thousands) with a focus on particular physiological or pathological condition and taking into consideration other contributing factors, such as environmental exposure and individual genetic and epigenetic makeup.

Sekovanic, A., et al. (2018). "Metallothionein 2A gene polymorphism and trace elements in mother-newborn pairs in the Croatian population." J Trace Elem Med Biol 45: 163-170.

The main source of exposure for all essential and toxic elements in the general population is diet. In smokers, the main route for cadmium (Cd) and lead (Pb) intake is the inhalation of tobacco smoke. Besides gender, age, nutrition, lifestyle, and physiological conditions such as pregnancy, specific genetic characteristics also influence individual element uptake. Metallothionein MT2 is a cysteine-rich low-weight protein found ubiquitously throughout the body. Specific gene polymorphism may influence MT2 expression and subsequent binding, transfer and organ accumulation of metals, though data on these influences are lacking, especially in human mother-newborn pairs. The objective of this study was to determine selected toxic (Cd, Pb, Hg) and essential (Fe, Zn, Cu, Se) elements in maternal blood, placenta, and cord blood (by ICP-MS), and MT2 levels in maternal serum (by ELISA) in relation to maternal MT2A -5A/G (rs28366003) polymorphism (by RFLP-PCR and electrophoresis). Study participants were healthy postpartum women in Croatia (n=268, mean age 29 years) with term vaginal childbirth in a maternity ward assigned into two study groups by self-reporting about their smoking habit (by questionnaire). Smokers vs. non-smokers had increased levels of Cd and Pb in all measured samples, Fe and Cu in cord blood, Zn in placenta, and MT2 in maternal serum. Among subjects with AG/GG genotype, placental Fe was significantly lower only among non-smokers, while MT2 levels in serum were lower, though not significantly, regardless of maternal smoking habit. There was no impact of MT2A -5A/G SNP on any element in maternal or cord blood. In conclusion, the results confirmed maternal smoking-related increases in Cd and Pb levels in the maternal-placental-foetal unit. They also provided additional data on concomitant metal concentrations in representative samples of maternal blood, placenta, and cord blood, as well as increased cord blood Fe and Cu, placental Zn, and maternal serum MT2 in smokers. New evidence is that MT2A -5A/G SNP was associated with decreased placental Fe levels in non-smokers. For a final conclusion on the influence of the MT2A -5A/G polymorphism on toxic and essential element levels in mother-newborn pairs, further research would require a larger number of participants divided across subgroups defined by the main source of particular toxic metal exposure (such as specific food intake, cigarette smoking, air pollution and/or occupational exposure).

Shanahan, C. M., et al. (1989). "Regulation of expression of a sheep metallothionein 1a-sheep growth hormone fusion gene in transgenic mice." Mol Cell Biol 9(12): 5473-5479.

Transgenic mice containing a sheep metallothionein 1a-sheep growth hormone fusion gene exhibited low, tissue-specific basal levels of transgene mRNA expression, resulting in slightly elevated levels of circulating growth hormone that did not lead to a detectable increase in growth. After zinc stimulation, high levels of transgene mRNA expression were induced in a number of tissues; these levels correlated with increased levels of circulating growth hormone, resulting in growth increases of up to 1.5 times the levels of controls and unstimulated transgenic mice. After removal of the zinc stimulus, transgene expression and circulating growth hormone concentrations returned to basal levels. Additional evidence from the pattern of developmental expression of the transgene suggests that zinc is the main regulator of this promoter in mice. The demonstrated regulation and low basal level of expression of the sheep metallothionein 1a promoter make it a candidate for use in other mouse transgenic studies and for use in transgenic livestock, in which regulation of expression is essential.

Shimizu, M., et al. (1998). "Effect of glutathione depletion and metallothionein gene expression on arsenic-induced cytotoxicity and c-myc expression in vitro." Toxicol Sci 45(2): 204-211.

Arsenic exposure is clearly linked to human cancer. In rodent cells, arsenic has been reported to induce aberrant gene expression, including activation of the proto-oncogene c-myc. Abnormal or altered expression of such oncogenes can be involved in the acquisition of a malignant phenotype. Although its mechanism of action is unclear, arsenic is known to exert at least some of its toxic effects through interaction with sulfhydryl groups, and the non-protein sulfhydryl glutathione (GSH) appears to play an important role in detoxication of arsenic. Similarly, metallothionein (MT), a metal-binding protein with high sulfhydryl content, often functions in defense against metal-induced or oxidative cellular injury. Therefore, we examined the relationship among GSH, MT gene expression, and arsenic-induced toxicity or c-myc expression in cultured rat myoblast (L6) cells. In initial toxicity studies, arsenic was used in both the trivalent (arsenite) and pentavalent (arsenate) forms. The role of GSH was studied by pretreating cells with L-buthionine sulfoximine (BSO), which induces a marked depletion of GSH. In vitro exposure of L6 cells to BSO (1 to 25 microM) resulted in dose-dependent decreases in GSH. GSH depletion sensitized cells to both arsenite and arsenate. Zinc pretreatment, at levels which highly activated MT expression, had no effect on arsenite-induced cytotoxicity. Arsenite (1 microM) alone modestly increased c-myc expression from 1 to 4 h after treatment (maximum of 2.0-fold over control). After GSH depletion cells responded to arsenite exposure with much larger increases in c-myc transcription (3.2-fold over control). Zinc pretreatment had no reductive effect on arsenite-induced c-myc expression despite markedly activating the MT gene. Thus, it appears that the cellular levels of GSH, but not MT gene expression, play an important role in resistance to arsenic toxicity and aberrant gene activation. Moreover, depletion of GSH enhances arsenic-induced proto-oncogene activation, which might contribute to subsequent transformation.

Singh, R. K., et al. (2011). "Metallothionein-like gene from Cicer microphyllum is regulated by multiple abiotic stresses." Protoplasma 248(4): 839-847.

Cicer microphyllum, a wild relative of cultivated chickpea, is a high altitude cold desert-adapted species distributed in western and trans-Himalayas. A complementary DNA (cDNA) encoding metallothionein-like protein has been identified from a cold-induced subtraction cDNA library from C. microphyllum. The sequence of the cloned metallothionein gene from C. microphyllum (GQ900702) contains 240-bp-long open reading frame and encodes predicted 79-amino acid protein of 7.9 kDa. Sequence analysis identified the motifs characteristic of type II metallothionein and designated as CmMet-2. Southern hybridization confirms a single copy of the CmMet-2 gene in C. microphyllum genome. In situ hybridization indicated spatial transcript regulation of CmMet-2 in root and aerial parts and also confirmed through real-time PCR-based quantitative transcript analysis. The data revealed a significantly low level of transcript in the aerial parts than the roots. Quantitative analysis using real-time PCR assay revealed induction of transcript in all parts of plants in response to cold stress at 4 degrees C. The transcript abundance was found to increase exponentially with time course from 6 to 24 h after exposure. Further, regulation of transcript accumulation in response to abscisic acid application, polyethylene glycol (100 muM)-induced osmotic stress, or ZnSO(4) (1 muM) foliar spray indicated by Northern hybridization suggests the involvement of CmMet-2 in multiple stress response.

Skroch, P., et al. (1993). "Regulation of human and yeast metallothionein gene transcription by heavy metal ions." Prog Clin Biol Res 380: 113-128.

Slater, E. P., et al. (1988). "Progesterone induction of metallothionein-IIA gene expression." Mol Endocrinol 2(6): 485-491.

Expression of the metallothionein gene is known to be induced by glucocorticoids in a variety of cells. Here we show that in human cell lines containing functional progesterone receptors, the endogenous metallothionein-IIA (hMTIIA) gene is inducible by the synthetic progestins R5020 and medroxy-progesterone acetate. That this effect reflects a direct interaction with the metallothionein gene is supported by our finding that the partially purified progesterone receptor binds to the promoter region of the gene in vitro. The limits of the DNase I footprint and the guanine residues protected in methylation studies with the progesterone receptor are similar to those previously described for the glucocorticoid receptor. Thus, the hormone regulatory element of the human metallothionein-IIA gene can mediate regulation by both glucocorticoids and progestins, as does the hormone regulatory element of mouse mammary tumor virus.

Smith, A., et al. (1993). "Regulation of heme oxygenase and metallothionein gene expression by the heme analogs, cobalt-, and tin-protoporphyrin." J Biol Chem 268(10): 7365-7371.

Two heme analogs, cobalt- and tin-protoporphyrin (CoPP and SnPP, respectively) have been used to probe the heme-hemopexin interaction, hemopexin receptor binding, and the mechanism of regulation of heme oxygenase (HO) and metallothionein-1 (MT-1) gene expression by hemopexin. Both CoPP and SnPP are HO inhibitors

and hemopexin binds SnPP (Morgan, W. T., Alam, J., Deaciuc, V., Muster, P., Tatum, F. M., and Smith, A. (1988) J. Biol. Chem. 263, 8226-8231) and CoPP. The association of CoPP with hemopexin produces characteristic changes in the absorbance spectrum of CoPP and quenches the intrinsic fluorescence of hemopexin. Binding of CoPP is tight (Kd ca. 3 x 10(-7) M) although of lower affinity than heme itself (Kd < pM); and CoPP binding, like heme, produces conformational changes in hemopexin shown by an increase in the molar ellipticity at 233 nm and affords protection from proteolysis of the hinge region between the two structural domains of hemopexin. The coordination of the central cobalt atom is predicted to be similar to that of heme and to involve His56 and His127 of rabbit hemopexin. Furthermore, CoPP-hemopexin, like SnPP-hemopexin, binds to the hemopexin receptor as shown by competitive inhibition studies with radioactive heme-hemopexin. The effect of free heme analogs and their hemopexin complexes on HO and MT gene regulation was investigated and compared with the extent of induction by heme and heme-hemopexin. Free CoPP is a more effective inducer of HO steady state mRNA levels than free heme and produces a 5-fold increase within 1 h compared to only a 2-fold increase with heme, but free SnPP (up to 10 microM) produces no detectable increase in HO mRNA. In contrast, by 3 h heme-hemopexin and SnPP-hemopexin increase HO mRNA levels 11- and 6-fold, respectively; but the CoPP-hemopexin complex causes no detectable change in HO mRNA levels. The complexes of hemopexin with heme or either of the two heme analogs are effective inducers of metallothionein (MT) mRNA. Induction of MT mRNA by heme-hemopexin is rapid, increasing 4-fold within 1 h and 14-fold by 3-4 h. Strikingly, an even more rapid and slightly more extensive induction of MT mRNA is seen in response to either CoPP- or SnPP-hemopexin complexes, with MT mRNA rising 8-fold within 1 h. In contrast, free heme and the free analogs are far less effective inducers, increasing MT and mRNA levels and in vitro transcription rates only 3-4-fold and declining after 2-3 h.(ABSTRACT TRUNCATED AT 400 WORDS)

Snibson, K. J., et al. (1995). "Methylation and expression of a metallothionein promoter ovine growth hormone fusion gene (MToGH1) in transgenic mice." Transgenic Res 4(2): 114-122.

We have examined transgene methylation in the DNA from the livers of a pedigree of mice carrying three copies of an integrated MToGH1 transgene. Utilizing the methylation-sensitive isoschizomers Msp I and Hpa II, Southern blot analysis revealed that all second generation animals derived from a transgenic female had hypermethylated DNA, whereas first generation animals sired by a transgenic male displayed a range of methylation phenotypes ranging from no methylation to hypermethylation of the transgene sequences. Of the mice that exhibited hypermethylation of the transgene in CpG dinucleotides (CmCGG), a minority of these animals also exhibited apparent CpC methylation (i.e. inhibition of Msp I cutting, presumably blocked by methylation of the outer C of CCGG). Methylation was also examined in the inner C of CC(A/T)GG sequences in the MToGH1 transgene using the isoschizomer pair BstN I and EcoR II. A minority of MToGH1 animals in the F1 generation showed clear evidence of methylation in these sites as well as in the inner and outer Cs of CCGG sites. An examination of MToGH1 expression in terms of oGH levels in serum revealed that there was a high degree of variation in the levels of circulating oGH between animals of this pedigree. There was a weak inverse relationship between the serum level of oGH and the extent of methylation of the transgene. In particular, mice exhibiting CpC together with CpG methylation were found to have very low levels of circulating oGH. Our results highlight the nature and complexity of epigenetic factors associated with transgene sequences which may ultimately influence expression of introduced genes in the mammalian genome.

Sode, K., et al. (1998). "Construction of a marine cyanobacterial strain with increased heavy metal ion tolerance by introducing exogenic metallothionein gene." J Mar Biotechnol 6(3): 174-177.

A marine cyanobacterial strain with enhanced heavy metal ion tolerance was constructed by introducing an exogenous cyanobacterial metallothionein gene, smtA, from a freshwater unicellular cyanobacterium, Synechococcus sp. PCC 7942. An expression vector harboring the c-phycocyanin (cpc) promoter and cpc N-terminal region was constructed and smtA was inserted into its multiple cloning site. The marine cyanobacterium, Synechococcus sp. NKBG 15041c, was highly sensitive to the heavy metal ions present in the medium. However, the recombinant marine cyanobacteria harboring the expression vector with smtA could grow even in the presence of 4 microM of CdCl2, at which concentration the wild-type strain did not grow.

Sogawa, N., et al. (2001). "The effects of ovariectomy and female sex hormones on hepatic metallothionein-I gene expression after injection of cadmium chloride in mice." Pharmacol Res 44(1): 53-57.

Metallothioneins (MTs) have a low molecular weight and have been considered to be important metal-binding proteins in defense from cadmium (Cd) toxicity in animals. These proteins are known to be induced by the injection of heavy metals such as Cd. Previously, we developed the measurement of the MT mRNA expression by the RT-PCR method. In this study,

to clarify the relation between Cd-induced MT-I mRNA expression and female sex hormones in liver, we investigated the influences of the ovariectomy and female sex hormones on hepatic MT-I mRNA expression after Cd injection, and also investigated the effects of aging on hepatic MT-I mRNA expression in mice after Cd injection. We analysed the MT-I mRNA expression by the RT-PCR method. Cd-induced MT-I mRNA expression in ovariectomized mice was more than that in sham-operated mice (9 weeks old). Both 17 beta -estradiol and progesterone reduced the Cd-induced MT-I mRNA expression in ovariectomized mice (9 weeks old). Moreover, the MT-I mRNA expression in male mice was more than that of females (9 weeks old). However, the sex difference in the gene expression was not found in younger (4 weeks old) or older (46 weeks old) mice. These results suggest that the expression of hepatic MT-I mRNA after Cd injection is influenced by female sex hormones.

Solis, W. A., et al. (2002). "Retrovirally expressed metal response element-binding transcription factor-1 normalizes metallothionein-1 gene expression and protects cells against zinc, but not cadmium, toxicity." Toxicol Appl Pharmacol 178(2): 93-101.

Metal response element (MRE) transcription factor-1 (MTF1), a member of the Cys2-His2 class of zinc-finger transcription factors, is best known for its robust transcriptional regulation of mammalian metallothionein (MT) genes. MTF1 is also believed to play a generalized role in regulating genes involved in protection against heavy metals and oxidative stress. MTF1 binding to MRE motifs is regulated by changes in intracellular zinc (Zn(2+)) concentration. Molecular dissection of MTF1 has been hindered by its high constitutive trans-activity following transient transfection and the failure of these systems to examine genes packaged in native chromatin. In developing a system to avoid these problems, we employed a high-efficiency retroviral transduction system to reintroduce MTF1 into mouse Mtf1(-/-) knockout cells (dko7). Electrophoretic mobility shift assays demonstrated that MTF1 retrovirally transduced dko7 cells (MTF1dko7) possess levels of inducible MTF1-MRE binding activity similar to that seen in mouse hepatoma Hepa-1 cells, and MTF1 binding could be modulated over a 20-fold range by varying the concentration of Zn(2+) present in the culture medium. The dko7 cells exhibited no change in Mt1 gene expression upon Zn(2+) or cadmium (Cd(2+)) treatment; in contrast, in MTF1dko7 cells, Zn(2+) or Cd(2+) induced MT1 mRNA accumulation in a dose-dependent manner. Interestingly, MTF1dko7 cells showed resistance to Zn(2+) toxicity, but negligible resistance to Cd(2+). Concomitantly, MT1 protein levels in MTF1dko7 cells were inducible to the same degree as that in Hepa-1 cells when treated with Zn(2+), but not with Cd(2+). Together, our studies suggest that MTF1-mediated regulation of gene expression is sufficient to protect cells against Zn(2+) toxicity and may be necessary but not sufficient to protect cells against Cd(2+) toxicity.

Somji, S., et al. (2001). "Metallothionein isoform 1 and 2 gene expression in the human bladder: evidence for upregulation of MT-1X mRNA in bladder cancer." Cancer Detect Prev 25(1): 62-75.

The goals of this study were to determine the expression of metallothionein isoform 1 and 2 proteins (MT-1 and MT-2) in bladder cancer and then to determine which MT isoform-specific genes promoted the expression of these proteins. Immunohistochemical analysis disclosed no immunoreactivity for MT-1 and MT-2 (designated as MT-1/2 to reflect the nonspecificity of the antibody for the two isoforms) in cells comprising the normal bladder or in nonmalignant bladder disorders, such as cystitis and interstitial cystitis. Immunohistochemical analysis demonstrated that MT-1/2 was overexpressed in all samples of carcinoma in situ and in high-grade bladder cancer, with variable overexpression in low-grade bladder cancer and dysplastic lesions. The intensity and frequency of MT-1/2 staining correlated with the grade of the tumor. The MT-1 and MT-2 proteins are encoded by a family of eight genes (MT-1A, MT-1B, MT-1E, MT-1F, MT-1G, MT-IH, MT-1X, and MT-2A), and reverse transcriptase-polymerase chain reaction was used to determine which genes were expressed in the normal bladder and in bladder cancer. This analysis demonstrated that both normal and cancerous bladder tissue expressed mRNA for the MT-2A and MT-1X genes. The expression of MT-1E mRNA was variable in both normal bladder and bladder cancer specimens. Comparison of expression relative to that of beta-actin demonstrated that the level of MT-1X mRNA was overexpressed greatly in bladder cancer as compared to the level in normal bladder tissue. In contrast, the level of MT-2A mRNA was similar in both the normal and the bladder cancer specimens. The level of MT-1X expression did not vary with tumor grade. These studies suggest that the overexpression of MT-1/2 protein in bladder cancer is a result of the overexpression of the MT-1X gene.

Stephan, W., et al. (1994). "Molecular evolution of the metallothionein gene Mtn in the melanogaster species group: results from Drosophila ananassae." Genetics 138(1): 135-143.

Three distinctly different alleles of the metallothionein gene Mtn have been identified in natural Drosophila melanogaster populations: Mtn.3, Mtn1, and Dp(Mtn1), where the latter designates a tandem duplication of Mtn1. In Drosophila simulans, only Mtn.3-type alleles have been found. It has been suggested that Mtn.3 is the ancestral allele and demonstrated that a presumed two-step transition from

Mtn.3 to Mtn1 to Dp(Mtn1) is accompanied by an approximate 5-fold increase in RNA levels. We analyzed the evolutionary genetics of the Mtn locus of Drosophila ananassae, a distant relative of D. melanogaster and D. simulans within the melanogaster species group. The Mtn gene of D. ananassae is most similar to Mtn.3: (i) it is identical with Mtn.3 at the amino acid level, but differs from Mtn1 in its terminal codon; (ii) its 3' UTR contains a characteristic extra DNA segment of about 50 bp which is present in Mtn.3, but lacking in Mtn1; (iii) duplications of Mtn were not found in a worldwide sample of 110 wild D. ananassae chromosomes. However, the intron of the Mtn gene in D. ananassae is only 69 bp long, whereas the length of the Mtn.3 and Mtn1 introns is 265 bp; and it lacks a polypyrimidine stretch upstream of the 3' splice site in contrast to the much greater pyrimidine-richness found in the Mtn.3 and Mtn1 introns. A short intron (67 bp) was also identified in a D. pseudoobscura Mtn allele, suggesting that the short intron is the ancestral form and that the transition from the short to the long intron occurred within the melanogaster species group.(ABSTRACT TRUNCATED AT 250 WORDS)

Stevens, M. E., et al. (1989). "Expression of a mouse metallothionein-Escherichia coli beta-galactosidase fusion gene (MT-beta gal) in early mouse embryos." Exp Cell Res 183(2): 319-325.

We have microinjected DNA containing the inducible mouse metallothionein-I (MT-I) promoter, coupled to the structural gene for Escherichia coli beta-galactosidase (lacZ), into the pronuclei of one-cell mouse embryos. A qualitative histochemical assay, with 5-bromo-4-chloro-3-indolyl beta-D-galactopyranoside (X-Gal) as a substrate, was used to detect expression of lacZ at several preimplantation stages. We observed staining indicative of exogenous beta-galactosidase activity in 5-17% of DNA-injected embryos assayed at preimplantation stages after 16-24 h treatment with ZnSO4. Thus, lacZ can be used as an indicator gene for promoter function during early mouse embryogenesis, and the incorporation of the MT-I promoter into fusion genes can be a useful means of controlling the expression of exogenous genes in preimplantation mouse embryos.

Stuart, G. W., et al. (1984). "A 12-base-pair DNA motif that is repeated several times in metallothionein gene promoters confers metal regulation to a heterologous gene." Proc Natl Acad Sci U S A 81(23): 7318-7322.

To define DNA sequences involved in mouse metallothionein-I (MT-I) gene promoter function and metal regulation, we fused the 5' flanking sequences of the MT-I gene to the coding sequences of a viral thymidine kinase (TK) gene. A series of 5' deletion, 3' deletion, linker-scanning, and internal deletion mutants of the MT-I promoter was constructed and assayed by microinjection into mouse eggs. The results indicate that at least two related promoter elements can confer some metal regulation independently. Those mutations that had the most severe effect on regulation impinge on a 12-base-pair conserved sequence that is repeated several times within the mouse MT-I and other MT promoters. To test the regulatory function of this sequence, it was synthesized as a pair of complementary oligonucleotides and inserted into the promoter of the TK gene. A single insertion of this sequence conferred limited metal regulation onto the TK promoter, whereas a construct with two separate inserts was regulated as efficiently as the MT-I promoter.

Sugimoto, M., et al. (1988). "Chemical synthesis and expression of copper metallothionein gene of Neurospora crassa." J Biochem 104(6): 924-926.

The gene coding for the Neurospora crassa copper metallothionein (MT) was synthesized and inserted in the lacZ' gene of pUC18 plasmid to give the same translational reading frame as the latter gene. The MT-beta-galactosidase fused gene was expressed in Escherichia coli to produce a fused protein in which the amino and carboxy termini of MT are linked to the beta-galactosidase through methionine residues. An MT derivative containing an extra homoserine residue at the carboxy terminus was prepared by cyanogen bromide cleavage of the fused protein followed by a reverse-phase HPLC separation. The spectral features of the MT derivative and its copper complex were similar to those of the corresponding native MTs.

Suh, M. C., et al. (1998). "Cadmium resistance in transgenic tobacco plants expressing the Nicotiana glutinosa L. metallothionein-like gene." Mol Cells 8(6): 678-684.

To understand the function of metallothioneins (MTs) in plants, we introduced the Nicotiana glutinosa MT gene into tobacco (N. tabacum) plants via an Agrobacterium mediated transformation. Full-length MT cDNA was fused between the cauliflower mosaic virus 35S (CaMV 35S) promoter and the nopaline synthase (nos) terminator of the pMBP1 binary vector in sense orientation. Tobacco leaf discs which were cocultivated with Agrobacterium carrying the chimeric MT gene, formed kanamycin-resistant shoots on medium containing kanamycin. The kanamycin-resistant shoots were subsequently rooted on medium containing 200 microM CdSO4. Approximately 30% of individual transgenic plants developed normally. Nontransgenic plants promptly underwent leaf chlorosis, and their growth and development were inhibited on MS medium containing 50 microM CdSO4. Genomic Southern blot analysis showed that the MT gene was stably integrated into the nuclear genome of transgenic tobacco plants. The expression level of MT transcripts was analyzed by RNA gel blot analysis. Self-pollinated seeds obtained from transgenic

tobacco plants showing cadmium tolerance were germinated on a medium containing 100 microM CdSO4. PCR analysis from sensitive and stably resistant T2 seedlings for cadmium sulfate confirmed a high correlation between the phenotypic expression of the MT gene and the transgenic genotype, indicating that the MT gene is inherited in the next generation.

Suleman, A. and A. R. Shakoori (2012). "Evaluation of physiological importance of metallothionein protein expressed by Tetrahymena cadmium metallothionein 1 (TMCd1) gene in Escherichia coli." J Cell Biochem 113(5): 1616-1622.

TMCd1 is a cadmium inducible metallothionein (MT) gene. In the present study the TMCd1 gene of a ciliate protozoan has been expressed in E. coli and the function of the expressed TMCd1 protein as a metal-binding protein has been evaluated. The growth of E. coli cells expressing the GST fused TMCd1 proteins in the presence of cadmium metal clearly demonstrated the role of TMCd1 as a metal-binding protein. The metal accumulation experiments showed that the bacterial cells expressing the functional TMCd1 protein accumulated 19-fold more cadmium in contrast to control cells that lacked the TMCd1 protein expression. The results clearly demonstrate a physiological role of full length TMCd1 protein of a ciliate, expressed in E. coli, in cadmium metal sequestration and detoxification.

Sun, W., et al. (2014). "Zinc rescue of Akt2 gene deletion-linked murine cardiac dysfunction and pathological changes is metallothionein-dependent." J Mol Cell Cardiol 74: 88-97.

We have demonstrated that zinc supplementation provides cardiac protection from diabetes in mice, but its underlying mechanism remains unclear. Since zinc mimics the function of insulin, it may provide benefit to the heart via stimulating Akt-mediated glucose metabolism. Akt2 plays an important role in cardiac glucose metabolism and mice with Akt2 gene deletion (Akt2-KO) exhibit a type 2 diabetes phenotype; therefore, we assumed that no cardiac protection by zinc supplementation from diabetes would be observed in Akt2-KO mice. Surprisingly, despite Akt2 gene deletion, zinc supplementation provided protection against cardiac dysfunction and other pathological changes in Akt2-KO mice, which were accompanied by significant decreases in Akt and GSK-3beta phosphorylation. Correspondingly, glycogen synthase phosphorylation and hexokinase II and PGC-1alpha expression, all involved in the regulation of glucose metabolism, were significantly altered in diabetic hearts, along with a significantly increased expression of Akt negative regulators: PTEN, PTP1B, and TRB3. All these molecular, pathological, and functional changes were significantly prevented by 3-month zinc supplementation. Furthermore, the stimulation of Akt-mediated glucose metabolic kinases or enzymes by zinc treatment was metallothionein-dependent since it could not be observed in metallothionein-knockout mice. These results suggest that zinc preserves cardiac function and structure in Akt2-KO mice presumably due to its insulin mimetic effect on cardiac glucose-metabolism. The cardioprotective effects of zinc are metallothionein-dependent. This is very important since zinc supplementation may be required for patients with Akt2 gene deficiency or insulin resistance.

Sun, Y., et al. (2004). "[Cloning, expression and purification of rabbit metallothionein-I gene in Escherichia coli]." Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 21(1): 76-80.

The cDNA encoding the rabbit metallothionein-I was amplified by RT-PCR from the rabbit liver induced by cadmium and cloned into prokaryotic fusion expression vector pQE40. Then it was transformed into Escherichia coli M15. Positive expression clones were detected by colony blotting. Target protein solubility was determined by Western blotting analysis. The optimal induction condition of the level of protein expression with IPTG induction was established by SDS-PAGE electrophoresis and ImageMaster VDS software analysis. The fusion protein can be purified from lysates with Ni-NTA agarose. We found that the fusion protein with apparent molecular weight 32 KD existed in two ways: soluble and insoluble in Escherichia coli. After 1 mM IPTG induction, the level of expression of the fusion protein increased with the prolongation of induction time and reached a peak in 9 h by ImageMaster VDS software analysis, accounting for 57.4% of all the insoluble protein. The purified fusion protein was obtained by Ni-NTA affinity chromatography. This fusion protein can be used in further studies on the preparation of MT-I protein and development of protein product.

Sutherland, G. R., et al. (1990). "The human metallothionein gene cluster is not disrupted in myelomonocytic leukemia." Genomics 6(1): 144-148.

The human metallothionein gene complex on chromosome 16 has been remapped to 16q13 using high-resolution in situ hybridization. The complex is not disrupted by the rearrangement breakpoint on the long arm of chromosome 16 in patients with myelomonocytic leukemia with abnormal eosinophils, as had been previously reported. The locus order on 16q is cen-MT-FRA16B-D16S4-inversion breakpoint-HP-tel.

Suzuki, K. and S. Koizumi (2000). "Individual metal responsive elements of the human metallothionein-IIA gene independently mediate responses to various heavy metal signals." Ind Health 38(1): 87-90.

Metallothioneins (MTs) are small metal-binding proteins that have a role in the defense against

heavy metals. Mammalian MT genes are transcriptionally activated by metals such as Cd and Zn through multiple copies of the metal responsive element (MRE) present in the 5'-flanking region. To examine whether each MRE in a single promoter has a distinct role, we characterized seven MREs located upstream of the human MT-IIA gene. By transient transfection experiments using MRE-driven reporter gene constructs, individual MREs were assayed for the activity to mediate transcription in response to several heavy metal species. Four MREs including MREs a, b, e and g independently mediated reporter gene expression in response to Zn, Cd and Hg, while other MREs were not responsive to any of these metals. These results suggest that the multiplicity of MRE contributes to enhancing its activity, rather than providing functional diversity.

Swain, P. S., et al. (2019). "Effect of Supplementation of Nano Zinc Oxide on Nutrient Retention, Organ and Serum Minerals Profile, and Hepatic Metallothionein Gene Expression in Wister Albino Rats." Biol Trace Elem Res 190(1): 76-86.

A study was conducted to validate the effects of nano form of zinc (NZn) on nutrient digestibility, zinc retention, organ and serum zinc profile, and hepatic metallothionein gene expression in Wistar albino rats (WAR). Nano zinc (NZn) was synthesized through chemical method, by using 0.45 M zinc nitrate [Zn(NO3)2.6H2O] and 0.9 M sodium hydroxide (NaOH). The NZn particle in its oxide form was characterized by TEM-EDAX and XRD, and found to be in nano range (below 100 nm. Zinc was supplemented to the Wistar albino rats (WAR) through synthetic semi-purified diet either without Zn, or as inorganic zinc (IZn; 25 mg/kg), or as synthesized NZn (25, 12.5, 6.25, 3.125 or 50 mg/kg DM) for 60 days. The zinc content was observed to be significantly ($P < 0.05$) higher in liver, bone, kidney, and serum due to NZn supplementation where NZn-50 had highest zinc content and control had the least, without affecting Fe, Mn, and Cu. NZn at 12.5 mg/kg group rats were either comparable or better than IZn at 25 mg/kg in terms of zinc retention, CP digestibility, zinc level in serum, liver, bone, and kidney suggesting its better bioavailability simultaneously also reduced fecal excretion of zinc to the environment. Metallothionein mRNA expression was upregulated in NZn at 25 mg/kg and NZn at 50 mg/kg than IZn at 50 mg/kg. Thus, in WAR, NZn at half of the ICAR recommendation (25 mg/kg DM) is as effective as inorganic zinc at 100% of recommended dose.

Szczypka, M. S. and D. J. Thiele (1989). "A cysteine-rich nuclear protein activates yeast metallothionein gene transcription." Mol Cell Biol 9(2): 421-429.

The ACE1 gene of the yeast Saccharomyces cerevisiae is required for copper-inducible transcription of the metallothionein gene (CUP1). The sequence of the cloned ACE1 gene predicted an open reading frame for translation of a 225-amino-acid polypeptide. This polypeptide was characterized by an amino-terminal half rich in cysteine residues and positively charged amino acids. The arrangement of many of the 12 cysteines in the configuration Cys-X-Cys or Cys-X-X-Cys suggested that the ACE1 protein may bind metal ions. The carboxyl-terminal half of the ACE1 protein was devoid of cysteines but was highly acidic in nature. The ability of a bifunctional ACE1-beta-galactosidase fusion protein to accumulate in yeast cell nuclei was consistent with the possibility that ACE1 plays a direct role in the regulation of copper-inducible transcription of the yeast metallothionein gene.

Taguchi, S., et al. (1998). "Identification of a structural gene encoding a metallothionein-like domain that includes a putative regulator protein for Streptomyces protease gene expression." Biosci Biotechnol Biochem 62(12): 2476-2479.

An open reading frame (termed ORF-PR) encoding a metallothionein-like domain-including protein was found upstream of a previously identified Streptomyces chymotrypsin-type protease gene (sam-P20). Promoter and terminator activities of ORF-PR were detected using the promoterless Streptomyces tyrosinase gene as a reporter gene and expression of ORF-PR was supposed to occur before that of sam-P20 gene. Frameshift mutation analysis showed that the ORF-PR product might act as a repressive regulator of the sam-P20 gene.

Takahashi, S. (2015). "Positive and negative regulators of the metallothionein gene (review)." Mol Med Rep 12(1): 795-799.

Metallothioneins (MTs) are metal-binding proteins involved in diverse processes, including metal homeostasis and detoxification, the oxidative stress response and cell proliferation. Aberrant expression and silencing of these genes are important in a number of diseases. Several positive regulators of MT genes, including metal-responsive element-binding transcription factor (MTF)-1 and upstream stimulatory factor (USF)-1, have been identified and mechanisms of induction have been well described. However, the negative regulators of MT genes remain to be elucidated. Previous studies from the group of the present review have revealed that the hematopoietic master transcription factor, PU.1, directly represses the expression levels of MT genes through its epigenetic activities, and upregulation of MT results in the potent inhibition of myeloid differentiation. The present review focuses on PU.1 and several other negative regulators of this gene, including PZ120, DNA methyltransferase 3a with Mbd3 and Brg1 complex, CCAAT enhancer binding protein alpha and Ku protein, and describes the suppression of the MT genes through

these transcription factors.
Tam, Y. C., et al. (1988). "Cloning, nucleotide sequence and characterization of a New Zealand rabbit metallothionein-I gene." Biochem Biophys Res Commun 153(1): 209-216.

We have isolated a rabbit metallothionein-I gene from a lambda gt10 library. The coding sequence of this gene is interrupted by two introns occurring at amino acid positions 9 1/3 and 30 1/3. Comparison of the promoter sequence of this gene with the promoters of other metallothionein genes identified a number of oligonucleotide sequences which are recognized by trans-acting proteins involved in the regulation of these genes by heavy metals, glucocorticoids and alpha interferon.

Tamai, K. T., et al. (1994). "Heat shock transcription factor activates yeast metallothionein gene expression in response to heat and glucose starvation via distinct signalling pathways." Mol Cell Biol 14(12): 8155-8165.

Metallothioneins constitute a class of low-molecular-weight, cysteine-rich metal-binding stress proteins which are biosynthetically regulated at the level of gene transcription in response to metals, hormones, cytokines, and other physiological and environmental stresses. In this report, we demonstrate that the Saccharomyces cerevisiae metallothionein gene, designated CUP1, is transcriptionally activated in response to heat shock and glucose starvation through the action of heat shock transcription factor (HSF) and a heat shock element located within the CUP1 promoter upstream regulatory region. CUP1 gene activation in response to both stresses occurs rapidly; however, heat shock activates CUP1 gene expression transiently, whereas glucose starvation activates CUP1 gene expression in a sustained manner for at least 2.5 h. Although a carboxyl-terminal HSF transcriptional activation domain is critical for the activation of CUP1 transcription in response to both heat shock stress and glucose starvation, this region is dispensable for transient heat shock activation of at least two genes encoding members of the S. cerevisiae hsp70 family. Furthermore, inactivation of the chromosomal SNF1 gene, encoding a serine-threonine protein kinase, or the SNF4 gene, encoding a SNF1 cofactor, abolishes CUP1 transcriptional activation in response to glucose starvation without altering heat shock-induced transcription. These studies demonstrate that the S. cerevisiae HSF responds to multiple, distinct stimuli to activate yeast metallothionein gene transcription and that these stimuli elicit responses through nonidentical, genetically separable signalling pathways.

Tang, C. M., et al. (1996). "Replication protein A is a component of a complex that binds the human metallothionein IIA gene transcription start site." J Biol Chem 271(35): 21637-21644.

Previous studies revealed that sequences surrounding the initiation sites in many mammalian and viral gene promoters, called initiator (Inr) elements, may be essential for promoter strength and for determining the actual transcription start sites. DNA sequences in the vicinity of the human metallothionein IIA (hMTIIA) gene transcription start site share homology with some of the previously identified Inr elements. However, in the present study we have found by in vitro transcription assays that the hMTIIA promoter does not contain a typical Inr. Electrophoretic mobility shift assays identified several DNA-protein complexes at the hMTIIA gene transcription start site. A partially purified protein fraction containing replication protein A (RPA) binds to the hMTIIA gene transcription start site and represses transcription from the hMTIIA promoter in vitro. In addition, overexpression of the human 70-kDa RPA-1 protein represses transcription of a reporter gene controlled by the hMTIIA promoter in vivo. These findings suggest that hMTIIA transcription initiation is controlled by a mechanism different from most mammalian and viral promoters and that the previously identified RPA may also be involved in transcription regulation.

Tang, C. M., et al. (1999). "trans repression of the human metallothionein IIA gene promoter by PZ120, a novel 120-kilodalton zinc finger protein." Mol Cell Biol 19(1): 680-689.

Metallothioneins are small, highly conserved, cysteine-rich proteins that bind a variety of metal ions. They are found in virtually all eukaryotic organisms and are regulated primarily at the transcriptional level. In humans, the predominant metallothionein gene is hMTIIA, which accounts for 50% of all metallothioneins expressed in cultured human cells. The hMTIIA promoter is quite complex. In addition to cis-acting DNA sequences that serve as binding sites for trans-acting factors such as Sp1, AP1, AP2, AP4, and the glucocorticoid receptor, the hMTIIA promoter contains eight consensus metal response element sequences. We report here the cloning of a novel zinc finger protein with a molecular mass of 120 kDa (PZ120) that interacts specifically with the hMTIIA transcription initiation site. The PZ120 protein is ubiquitously expressed in most tissues and possesses a conserved poxvirus and zinc finger (POZ) motif previously found in several zinc finger transcription factors. Intriguingly, we found that a region of PZ120 outside of the zinc finger domain can bind specifically to the hMTIIA DNA. Using transient-transfection analysis, we found that PZ120 repressed transcription of the hMTIIA promoter. These results suggest that the hMTIIA gene is regulated by an additional negative regulator that has not been previously described.

Tanguy, A. and D. Moraga (2001). "Cloning and characterization of a gene coding for a novel metallothionein in the Pacific oyster Crassostrea gigas

(CgMT2): a case of adaptive response to metal-induced stress?" Gene 273(1): 123-130.

Cases of heavy metal resistance acquisition have already been demonstrated in eukaryotes, which involve metallothionein (MT) gene duplication or amplification mechanisms. We characterized in a marine bivalve, Crassostrea gigas, a gene coding for an unusual MT, which has never been described in other species. Our results illustrate a unique case of exon duplication and rearrangement in the MT gene family. The particular organization of the third exon of this gene allows the synthesis of a MT that presents a higher metal ion binding capacity compared to previously described MTs. The formation of a supplementary third structural beta-domain is proposed to explain results obtained in in vitro experiments. Differences in the metal responsive element (MRE) copy number and MRE core sequence observed in the promoter of CgMT2 also suggest differential regulation of CgMT2 transcription and possible implication in the detoxification processes.

Tanguy, A., et al. (2001). "Cloning of a metallothionein gene and characterization of two other cDNA sequences in the Pacific oyster Crassostrea gigas (CgMT1)." Aquat Toxicol 55(1-2): 35-47.

Metallothionein (MT) genes encode essential metal-binding proteins involved in metallic homeostasis and detoxification in living organisms. Here, we describe the structure of the first Pacific oyster Crassostrea gigas metallothionein (CgMT1) gene and the sequences of two other MT cDNA. The CgMT1 gene sequence contains three coding exons plus a 5' entirely non-coding exon, and the predicted protein contains 21 cysteine residues organized in Cys-X-Cys motifs as classically described for MTs. The three cDNA sequences present few substitutions in either coding sequence or UTRs. Induction of these MT-mRNA in heavy metal-treated oysters (i.e. cadmium) was confirmed by Northern blot analysis and RT-PCR and suggests a potential specific tissue expression rate. Southern blot analysis suggested the presence of multiple CgMT genes, and allowed the detection of restriction fragment length polymorphisms (RFLPs). Although the CgMT1 coding sequence showed 30-73% nucleotide identities with known sequences in other mollusks, it included the specific motif Cys-X-Cys-X(3)-Cys-Thr-Gly-X-X-X-Cys-X-Cys-X(5)-Cys-X-Cys-Lys found in Mollusk family 2. Marine bivalves are commonly used as pollution bioindicators, thus the development of genetic markers based on CgMT1 polymorphism will allow a monitoring of heavy metal exposure in anthropogenically disturbed ecosystems.

Tanuma, S., et al. (1987). "Evidence of a regulatory role of the level of poly (ADP-ribose) in chromosomal proteins in metallothionein gene expression by glucocorticoids but not by heavy metals." Biochim Biophys Acta 910(2): 197-201.

The induction capacity of dexamethasone, a synthetic glucocorticoid, for the synthesis of metallothionein was about the same as that of 3-aminobenzamide, which is an inhibitor of ADP-ribosylation of chromosomal proteins, in cultured mouse mammary tumor cells. Both inductions of metallothionein were temporally correlated with a decrease in the amount of endogenous poly (ADP-ribose) on nonhistone high-mobility-group 14 and 17 proteins. In contrast, the extent of cadmium-induced metallothionein synthesis was 2-3-times that of dexamethasone or 3-aminobenzamide. However, cadmium had essentially no effect on de-ADP-ribosylation of these proteins.

Tao, Y. F., et al. (2014). "Metallothionein III (MT3) is a putative tumor suppressor gene that is frequently inactivated in pediatric acute myeloid leukemia by promoter hypermethylation." J Transl Med 12: 182.

BACKGROUND: Acute myeloid leukemia (AML) is the second most common form of leukemia in children. Aberrant DNA methylation patterns are a characteristic feature in various tumors, including AML. Metallothionein III (MT3) is a tumor suppresser reported to show promoter hypermethylated in various cancers. However, the expression and molecular function of MT3 in pediatric AML is unclear. METHODS: Eleven human leukemia cell lines and 41 pediatric AML samples and 20 NBM/ITP (Norma bone marrow/Idiopathic thrombocytopenic purpura) control samples were analyzed. Transcription levels of MT3 were evaluated by semi-quantitative and real-time PCR. MT3 methylation status was determined by methylation specific PCR (MSP) and bisulfite genomic sequencing (BSG). The molecular mechanism of MT3 was investigated by apoptosis assays and PCR array analysis. RESULTS: The MT3 promoter was hypermethylated in leukemia cell lines. More CpG's methylated of MT3 was observed 39.0% pediatric AML samples compared to 10.0% NBM controls. Transcription of MT3 was also significantly decreased in AML samples compared to NBM/ITP controls ($P < 0.001$); patients with methylated MT3 exhibited lower levels of MT3 expression compared to those with unmethylated MT3 ($P = 0.049$). After transfection with MT3 lentivirus, proliferation was significantly inhibited in AML cells in a dose-dependent manner ($P < 0.05$). Annexin V assay showed that apoptosis was significantly upregulated MT3-overexpressing AML cells compared to controls. Real-time PCR array analysis revealed 34 dysregulated genes that may be implicated in MT3 overexpression and apoptosis in AML, including FOXO1. CONCLUSION: MT3 may be a putative tumor suppressor gene in pediatric AML. Epigenetic inactivation of MT3 via promoter

hypermethylation was observed in both AML cell lines and pediatric AML samples. Overexpression of MT3 may inhibit proliferation and induce apoptosis in AML cells. FOXO1 was dysregulated in MT3-overexpressing cells, offering an insight into the mechanism of MT3-induced apoptosis. However, further research is required to determine the underlying molecular details.

Taplitz, S. J., et al. (1986). "Alternative inducers of the rat metallothionein I gene cause distinct changes in chromatin structure in the 5' region of the gene." Mol Cell Biol 6(7): 2576-2581.

We examined the chromatin structure of the rat metallothionein I gene, both in uninduced cells and in cells induced by heavy metals or dexamethasone, using hypersensitivity to DNase I as an assay. The metallothionein I gene of the H4IIE rat hepatoma cell line, expressed at basal level, has a single DNase I-hypersensitive site. This site maps between putative hormone and basal level control sequences. Induction of the gene by cadmium or zinc resulted in the appearance of a new hypersensitive site near the start site of transcription, in a region near the metal-regulatory elements. In contrast, induction of the metallothionein I gene by dexamethasone did not alter the basal pattern of hypersensitivity. Thus, different mechanisms of induction of metallothionein transcription lead to distinct alterations in the chromatin containing the 5' sequences regulating the expression of this gene.

Tate, D. J., Jr., et al. (1995). "Phagocytosis and H2O2 induce catalase and metallothionein gene expression in human retinal pigment epithelial cells." Invest Ophthalmol Vis Sci 36(7): 1271-1279.

PURPOSE: Reactive oxygen intermediates have been implicated in the aging process and degenerative diseases of the eye, including retinopathy of prematurity, cataractogenesis, and macular degeneration. The purpose of this study was to investigate the effect of phagocytosis of photoreceptor outer segments and the addition of exogenous H2O2 on catalase and metallothionein expression in human retinal pigment epithelial cells. METHODS: Confluent RPE cells were treated with bovine photoreceptor outer segments or H2O2 for either 6 or 18 hours. Slot blot hybridization was used to assess catalase and metallothionein gene expression after 6 hours. Catalase enzyme activity and metallothionein content were measured after 18 hours. RESULTS: Phagocytosis or the addition of H2O2 increased catalase enzyme activity and metallothionein twofold above control levels. The addition of n-acetyl cysteine abrogated the inductive effect caused by either stress. Catalase and metallothionein gene expression, measured by slot blot hybridization, also were measurably induced by either stress. Phagocytosis of photoreceptor outer segments increased extracellular H2O2 concentration nine times above control. CONCLUSIONS: The response of the retinal pigment epithelial cells to phagocytosis was indistinguishable from the response observed after the addition of exogenous H2O2. The generation of H2O2 during phagocytosis may act as an intracellular signal in retinal pigment epithelial cells that leads to increased levels of key antioxidant enzymes and other proteins important for protecting the cells from oxidative damage.

Tennekoon, G. I., et al. (1987). "Transfection of neonatal rat Schwann cells with SV-40 large T antigen gene under control of the metallothionein promoter." J Cell Biol 105(5): 2315-2325.

Secondary cultures of Schwann cells were transfected with a plasmid containing the SV-40 T antigen gene expressed under the control of the mouse metallothionein-I promoter. We used the calcium phosphate method for transfection and obtained a transfection efficiency of 0.01%. The colonies were cloned by limited dilution, and these cloned cell lines were carried in medium containing zinc chloride (100 microM). One cloned cell line, which has now been carried for 180 doublings, appears to have a transformed phenotype with a doubling time of 20 h. These cells express SV-40 T antigen while maintaining established Schwann cell properties (positive staining for 217c, Ran-2, A5E3, glial fibrillary acidic protein, presence of 2',3'-cyclic nucleotide phosphohydrolase [CNPase] activity, and the ability to synthesize sulfogalactosylceramide and mRNA for the myelin protein, P0). Removal of zinc chloride from the medium resulted in reduced expression of T antigen and a change in the appearance of the cells to a more bipolar shape, although they still did not exhibit contact inhibition and maintained a doubling time of 20 h. These cells now became Ran-2-negative and showed increases in CNPase activity and in their ability to synthesize sulfogalactosylceramide. The amount of P0 mRNA remained unchanged. Transfected Schwann cells, however, stopped dividing when they contacted either basal lamina or neurites and became bipolar in appearance. The Schwann cells in contact with the neurites then extended processes to wrap around bundles of neurites. Transfection with the SV-40 T antigen gene therefore provides a method for obtaining Schwann cell lines that continue to express properties associated with untransfected cells in culture and may be used to study axon-Schwann cell interaction.

Theodore, L., et al. (1991). "Recent evolutionary history of the metallothionein gene Mtn in Drosophila." Genet Res 58(3): 203-210.

A new allele of one of the metallothionein genes of D. melanogaster, Mtn.3, sheds light on the recent evolution of this gene. In comparison to the

previously studied Mtn1 allele found in Canton S, this new allele, Mtn.3, produces a transcript that is 49 bases longer and 65-70% less abundant. We detected Mtn.3 in several laboratory strains as well as in isofemale lines derived from natural populations. Sequence comparison showed that Mtn.3 differs from Mtn1 in that it has: (a) base-pair substitution and an extra 49 bp-segment in the 3' untranslated region, (b) a substitution in the coding region that replaces the terminal Glu40 in Mtn1 with Lys40, and (c) two base-pair substitutions in the promoter region. The Mtn.3-type was detected in six species of the melanogaster group by restriction analysis, and this result was confirmed by sequencing the D. simulans Mtn gene. Thus Mtn.3, which produces a less abundant transcript, appears to be the oldest of the two alleles. We also found that the duplications previously isolated from natural populations all derived from Mtn1, the more recent allele. Thus, two evolutionary steps: Mtn.3 to Mtn1 and Mtn1 to Dp(Mtn1), are accompanied by an overall 5- to 6-fold increase of RNA accumulation. The two changes seem to have occurred in non-African populations since Mtn.3 but not Mtn1 was detected in our sample from tropical Africa, while Mtn1 and Dp (Mtn1) are prevalent in European and North American samples.

Thiele, D. J. (1988). "ACE1 regulates expression of the Saccharomyces cerevisiae metallothionein gene." Mol Cell Biol 8(7): 2745-2752.

Copper resistance in Saccharomyces cerevisiae is mediated, in large part, by the CUP1 locus, which encodes a low-molecular-weight, cysteine-rich metal-binding protein. Expression of the CUP1 gene is regulated at the level of transcriptional induction in response to high environmental copper levels. This report describes the isolation of a yeast mutant, ace1-1, which is defective in the activation of CUP1 expression upon exposure to exogenous copper. The ace1-1 mutation is recessive and lies in a genetic element that encodes a trans-acting CUP1 regulatory factor. The wild-type ACE1 gene was isolated by in vivo complementation and restores copper inducibility of CUP1 expression and copper resistance to the otherwise copper-sensitive ace1-1 mutant. Linkage analysis and gene deletion experiments verified that this gene represents the authentic ACE1 locus. ACE1 maps to the left arm of chromosome VII, 9 centimorgans centromere distal to lys5. The ACE1 gene appears to play a direct or indirect positive role in activation of CUP1 expression in response to elevated copper concentrations.

Thiele, D. J. and D. H. Hamer (1986). "Tandemly duplicated upstream control sequences mediate copper-induced transcription of the Saccharomyces cerevisiae copper-metallothionein gene." Mol Cell Biol 6(4): 1158-1163.

Transcription of the Saccharomyces cerevisiae copper-metallothionein gene, CUP1, inducible by copper. By analyzing deletion and fusion mutants in the CUP1 5'-flanking region, we identified two closely related, tandemly arranged copper regulatory elements. A synthetic version of one of these elements conferred efficient copper induction on a heterologous promoter when present in two tandem copies.

Thummabancha, K., et al. (2016). "Analysis of hematologic alterations, immune responses and metallothionein gene expression in Nile tilapia (Oreochromis niloticus) exposed to silver nanoparticles." J Immunotoxicol 13(6): 909-917.

In this study, Nile tilapia (Oreochromis niloticus) fingerlings were used as a model to examine acute and chronic toxicity of silver nanoparticles (AgNP). Expression levels of metallothionein (MT) transcripts in fish exposed to 0, 1 or 100 mg AgNP/kg fish were investigated by quantitative real-time RT-PCR. The results showed MT expression levels were significantly decreased 0.3-0.7-fold in the liver and spleen of fish exposed to 1 or 100 mg AgNP/kg after 6-48 h. In contrast, during this period, MT mRNA expression levels were increased 2-3-fold in the head kidney of the fish exposed to either level of AgNP. Investigations of effects of AgNP on the fish immune responses and hematological parameters revealed that phagocytic activity, the amount of red blood cells (RBC) and the percent hematocrit (%Hct) in fish exposed to AgNP were decreased significantly 1 week after exposure, especially those exposed to 100 mg AgNP/kg. Fish immunized with Streptococcus agalactiae vaccine and simultaneously exposed to 100 mg AgNP/kg presented decreased antibody titers during the early phase. Lastly, a challenge test showed that vaccinated fish exposed to AgNP, regardless of concentration, remained protected against S. agalactiae infection, with a lower mortality (10-20%) compared to 70% in control fish. These findings indicated that expression patterns of the MT gene in the liver, spleen and head kidney at different timepoints could be used to assess acute and chronic exposure of Nile tilapia to AgNP. Additionally, changes in innate immune responses and hematological parameters in fish may prove useful for evaluation of AgNP toxicity. Data obtained in this study strongly support the use of Nile tilapia as an animal model to potentially serve as a bio-indicator of environment contamination caused by AgNP.

Tian, Z. Q., et al. (2013). "Effects of metallothionein-3 and metallothionein-1E gene transfection on proliferation, cell cycle, and apoptosis of esophageal cancer cells." Genet Mol Res 12(4): 4595-4603.

Metallothionein (MT)-3 has cell growth inhibitory activity, and is the only currently known MT subtype with unique physiological functions. The expression levels of MT-1E, a subtype of MT-1, were

positively correlated with the degree of esophageal cancer malignancy. The present study aimed to investigate the effects of MT-3 and MT-1E gene transfection on the proliferation, cell cycle, and apoptosis of esophageal cancer cells. The cationic liposome method was used to transfect the esophageal cancer strains Eca-109 and TE13. Reverse transcription-polymerase chain reaction was used to detect target gene expression, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction was applied to detect cell proliferation, and flow cytometry was used for cell cycle and apoptosis detection. Esophageal cancer cells with MT-3 and MT-1E gene transfection showed high expression of the foreign target gene and mRNA. Cells with MT-3 gene transfection showed markedly inhibited proliferation ($P < 0.05$), a significantly higher proportion of cells in the G0/G1 phase ($P < 0.05$), a significantly lower proportion of cells in the S phase ($P < 0.05$), and a significantly increased apoptosis rate ($P < 0.05$). Cells with MT-1E gene transfection did not show significant changes in proliferation, cell cycle, or apoptosis rate ($P > 0.05$). Therefore, the upregulation of MT-3 gene expression can inhibit esophageal cancer cell proliferation and induce apoptosis, which may be achieved by blocking the tumor cell growth cycle, whereas effects of the MT-1E gene on the proliferation of esophageal cancer cells were not evident.

Tio, L., et al. (2004). "Functional differentiation in the mammalian metallothionein gene family: metal binding features of mouse MT4 and comparison with its paralog MT1." J Biol Chem 279(23): 24403-24413.

This paper reports on the characterization of the metal binding abilities of mammalian MT4 and their comparison with those of the well known MT1. Heterologous Escherichia coli expression in cultures supplemented with zinc, cadmium, or copper was achieved for MT4 and for its separate alphaMT4 and betaMT4 domains as well as for MT1 and its alphaMT1 domain in cadmium-enriched medium. The in vivo conformed metal complexes and the in vitro substituted zinc/cadmium and zinc/copper MT4 aggregates were characterized. Biosynthesis of MT4 and betaMT4 in Cd(II)-supplemented medium revealed that these peptides failed to form the same homometallic species as MT1, thus appearing less effective for cadmium coordination. Conversely, the entire MT4 and both of its domains showed better Cu(I) binding properties than MT1, affording Cu(10)-MT4, Cu(5)-alphaMT4 and Cu(7)-betaMT4, stoichiometries that make the domain dependence toward Cu(I) clear. Overall results allow consideration of MT4 as a novel copper-thionein, made up of two copper-thionein domains, the first of this class reported in mammals, and by extension in vertebrates. Furthermore, the in silico protein sequence analyses corroborated the copper-thionein nature of the MT4 peptides. As a consequence, there is the suggestion of a possible physiological role played by MT4 related with copper requirements in epithelial differentiating tissues, where MT4 is expressed.

Tohoyama, H., et al. (1992). "Constitutive transcription of the gene for metallothionein in a cadmium-resistant yeast." FEMS Microbiol Lett 74(1): 81-85.

A cadmium-resistant strain of Saccharomyces cerevisiae produces a cadmium metallothionein encoded by the CUP1 gene as does a copper-resistant strain. The mechanism of expression of the gene is inducible by copper ions in the copper-resistant strain. However, assays of CUP1-specific mRNA revealed that the transcription of the CUP1 gene in the cadmium-resistant strain is constitutive and the rate of transcription is further increased by exposure to cadmium or copper ions. This result was confirmed by the appearance of constitutive-expression segregants from diploid crosses between the cadmium-resistant strain and a strain with a reporter gene having the promoter of CUP1.

Tohoyama, H., et al. (2001). "Induction for the expression of yeast metallothionein gene, CUP1, by cobalt." Microbios 104(408): 99-104.

Induction for the expression of the metallothionein gene, CUP1, in the yeast Saccharomyces cerevisiae by cobalt was examined using a reporter gene with the promoter of this gene fused to the coding region of lacZ. The expression of the gene was induced by cobalt as well as by copper and silver ions. The activity of beta-galactosidase showed high levels after treatment with 1.0 mM cobalt chloride. It has been reported that the induction for the transcription of CUP1 by copper and silver is mediated by the Ace1 transcription factor. However, the expression of the gene by cobalt occurred in yeast cells lacking the Ace1 factor. These results suggest the presence of a novel cobalt-specific transcription factor for the CUP1 gene.

Tohoyama, H., et al. (1992). "The gene for cadmium metallothionein from a cadmium-resistant yeast appears to be identical to CUP1 in a copper-resistant strain." Curr Genet 21(4-5): 275-280.

A cadmium-resistant strain of Saccharomyces cerevisiae produces a cadmium metallothionein with the same characteristics as the copper metallothionein that is encoded by CUP1 in a copper-resistant strain. The structural gene for metallothionein from the cadmium-resistant strain resembles CUP1 in terms of the fragmentation patterns generated by restriction enzymes. Furthermore, the gene may be amplified as 2.0 kb repeating units in both the cadmium-resistant and the copper-resistant strains. However, transformants with a plasmid that carried the metallothionein gene from the cadmium-resistant strain

were resistant to copper but not to cadmium. It appears that the same metallothionein gene, CUP1, is amplified in both cadmium- and copper-resistant yeasts. However, the mechanism for the cadmium-specific inducibility of the gene may be restricted to the cadmium-resistant strain.

Townes, T. M., et al. (1985). "Expression of human beta-globin genes in transgenic mice: effects of a flanking metallothionein-human growth hormone fusion gene." Mol Cell Biol 5(8): 1977-1983.

In an attempt to place a human beta-globin gene in an open chromatin domain regardless of its site of integration in the mouse genome, we microinjected into fertilized mouse eggs a construct in which the human beta-globin gene and a mouse metallothionein-human growth hormone fusion gene were juxtaposed and oriented in opposite directions. Mice that developed from injected eggs and that grew larger than normal were analyzed for human beta-globin mRNA. The globin genes were not expressed in erythroid tissue but were expressed with the same tissue specificity as metallothionein-human growth hormone. These results suggest that sequences which control metallothionein-human growth hormone gene expression are capable of stimulating the expression of a flanking gene in an orientation-independent and tissue-specific manner. As a control for this experiment, we deleted the metallothionein-human growth hormone transcription unit and noted that the human beta-globin gene then was expressed at high levels with erythroid tissue specificity.

Trayhurn, P., et al. (2000). "Metallothionein gene expression and secretion in white adipose tissue." Am J Physiol Regul Integr Comp Physiol 279(6): R2329-2335.

White adipose tissue (WAT) has been examined to determine whether the gene encoding metallothionein (MT), a low-molecular-weight stress response protein, is expressed in the tissue and whether MT may be a secretory product of adipocytes. The MT-1 gene was expressed in epididymal WAT, with MT-1 mRNA levels being similar in lean and obese (ob/ob) mice. MT-1 mRNA was found in each of the main adipose tissue sites (epididymal, perirenal, omental, subcutaneous), and there was no major difference between depots. Separation of adipocytes from the stromal-vascular fraction of WAT indicated that the MT gene (MT-1 and MT-2) was expressed in adipocytes themselves. Treatment of mice with zinc had no effect on MT-1 mRNA levels in WAT, despite strong induction of MT-1 expression in the liver. MT-1 gene expression in WAT was also unaltered by fasting or norepinephrine. However, administration of a beta(3)-adrenoceptor agonist, BRL-35153A, led to a significant increase in MT-1 mRNA. On differentiation of fibroblastic preadipocytes to adipocytes in primary culture, MT was detected in the medium, suggesting that the protein may be secreted from WAT. It is concluded that WAT may be a significant site of MT production; within adipocytes, MT could play an antioxidant role in protecting fatty acids from damage.

Trayhurn, P., et al. (2000). "Regulation of metallothionein gene expression and secretion in rat adipocytes differentiated from preadipocytes in primary culture." Horm Metab Res 32(11-12): 542-547.

The gene encoding metallothionein, a low mol. wt. metal binding and stress response protein, is expressed in white adipose tissue. In the present study, metallothionein (MT-1) gene expression and factors regulating metallothionein production have been examined in adipocytes induced to differentiate from fibroblastic preadipocytes in primary cell culture. On the induction of differentiation, the metallothionein-1 gene was strongly expressed in the cells and metallothionein released into the medium. A peak in metallothionein-1 mRNA level and metallothionein secretion occurred at 2 and 10 days post-differentiation, respectively, with a decrease in protein release after this time. The metallothionein-1 gene was expressed in the adipocytes prior to the adipsin and lipoprotein lipase genes, suggesting that it is an early marker of adipocyte differentiation. The addition of the glucocorticoid, dexamethasone, led to a substantial increase in metallothionein-1 mRNA in the cells and metallothionein secretion. Insulin and leptin also stimulated metallothionein production, although the effect was small. Neither noradrenaline nor the beta3-adrenoceptor agonist, BRL 37 344, altered metallothionein release but forskolin and bromo-cAMP were stimulatory, markedly increasing both metallothionein-1 level and metallothionein secretion. It is suggested that metallothionein is a novel secretory product of the differentiated white adipocyte and that its production is regulated particularly by glucocorticoids and through a cAMP-dependent pathway.

Trendelenburg, G., et al. (2002). "Serial analysis of gene expression identifies metallothionein-II as major neuroprotective gene in mouse focal cerebral ischemia." J Neurosci 22(14): 5879-5888.

We applied serial analysis of gene expression (SAGE) to study differentially expressed genes in mouse brain 14 hr after the induction of focal cerebral ischemia. Analysis of >60,000 transcripts revealed 83 upregulated and 94 downregulated transcripts (more than or equal to eightfold). Reproducibility was demonstrated by performing SAGE in duplicate on the same starting material. Metallothionein-II (MT-II) was the most significantly upregulated transcript in the ischemic hemisphere. MT-I and MT-II are assumed to be induced by metals, glucocorticoids, and inflammatory signals in a coordinated manner, yet their

function remains elusive. Upregulation of both MT-I and MT-II was confirmed by Northern blotting. MT-I and MT-II mRNA expression increased as early as 2 hr after 2 hr of transient ischemia, with a maximum after 16 hr. Western blotting and immunohistochemistry revealed MT-I/-II upregulation in the ischemic hemisphere, whereas double labeling demonstrated the colocalization of MT with markers for astrocytes as well as for monocytes/macrophages. MT-I- and MT-II-deficient mice developed approximately threefold larger infarcts than wild-type mice and a significantly worse neurological outcome. For the first time we make available a comprehensive data set on brain ischemic gene expression and underscore the important protective role of metallothioneins in ischemic damage of the brain. Our results demonstrate the usefulness of SAGE to screen functionally relevant genes and the power of knock-out models in linking function to expression data generated by high throughput techniques.

Tschuschke, S., et al. (2002). "Cadmium resistance conferred to yeast by a non-metallothionein-encoding gene of the earthworm Enchytraeus." J Biol Chem 277(7): 5120-5125.

The earthworm Enchytraeus is able to survive in cadmium (Cd)-polluted environments. Upon Cd exposure, the worms express a gene encoding the putative non-metallothionein 25-kDa cysteine-rich protein (CRP), which contains eight repeats with highly conserved cysteines in Cys-X-Cys and Cys-Cys arrangements exhibiting 36-53% identities to the 6-7-kDa metallothioneins of different organisms. Here, we demonstrate that the CRP protein confers a highly Cd-resistant phenotype to a Cd-hypersensitive yeast strain. Cd resistance increases with increasing numbers of expressed CRP repeats, but even one 3-kDa CRP repeat still mediates Cd resistance. Site-directed mutagenesis reveals that each single cysteine within a given repeat is important for Cd resistance, though to a different extent. However, replacement of other conserved amino acids such as Pro(136) and Asp(196) at the CRP repeat junctions does not affect Cd resistance. Our data indicate (i) that the non-metallothionein CRP protein is able to detoxify Cd and (ii) that this is dependent on the availability of sulfhydryl groups of the conserved cysteines.

Tse, K. Y., et al. (2009). "Epigenetic alteration of the metallothionein 1E gene in human endometrial carcinomas." Tumour Biol 30(2): 93-99.

Aberrant expression of metallothioneins (MTs) has been observed in several human tumors. In our microarray analysis, MT-1E was found to have much lower expression in endometrial cancer cells as compared with other types of cancer cells generated from the cervix, ovary or prostate. The result was confirmed by quantitative RT-PCR analysis of the MT-1E levels in individual cancer cells. Treatment of endometrial cancer cells with 5-azacytidine could reactivate MT-1E expression. We further analyzed the DNA methylation status of the promoter region of MT-1E using methylation-sensitive restriction enzymes HhaI and HpaII, followed by PCR. Promoter hypermethylation was detected in 42.4% (53/125) of the endometrial carcinoma samples, whilst none of the 38 normal tissues or hyperplasia samples were methylated. The mRNA levels of MT-1E were significantly lower in the methylation-positive than in the methylation-negative samples. Endometrial carcinoma samples with low MT-1E expression coincidently had low levels of estrogen receptor-alpha expression and vice versa. This phenomenon was not observed in the expression pattern between estrogen receptor-beta and MT-1E. There was no significant correlation between MT-1E methylation and any clinical parameters. In conclusion, a high frequency of cancer-specific hypermethylation of MT-1E was found in endometrial carcinomas. Its functional consequence in the development of endometrial cancer warrants further investigation.

Turchi, A., et al. (2012). "Expression of a metallothionein A1 gene of Pisum sativum in white poplar enhances tolerance and accumulation of zinc and copper." Plant Sci 183: 50-56.

Metallothioneins (MT) play an important role in heavy metal detoxification and homeostasis of intracellular metal ions in plant. In this study, two transgenic lines expressing MT type 2 gene (PsMT(A1)) from Pisum sativum, a regenerated non transformed line NT and clone AL22, selected as heavy metal tolerant, were characterized in presence of the heavy metals for the ability to accumulate zinc and copper and to activate antioxidative enzyme defences: superoxide dismutase, catalase, ascorbate peroxidase. The levels of expression of MT type 2 gene assessed by RT-qPCR confirmed the gene over-expression in transgenic lines and evidenced in NT and AL22 the up-regulation of gene transcription by zinc and copper. Transgenic poplar lines during heavy metal stress displayed increased ability to translocate and accumulate zinc and copper compared with NT and AL22. The antioxidant enzyme defence was differently activated in response to metals in the transgenic lines without a significant increase of ROS. These results suggested that PsMT(A1) could play a role in ROS scavenging leading to enhanced metal tolerance and increased zinc and copper sequestration in root and leaf.

van Hoof, N. A., et al. (2001). "Enhanced copper tolerance in Silene vulgaris (Moench) Garcke populations from copper mines is associated with increased transcript levels of a 2b-type metallothionein gene." Plant Physiol 126(4): 1519-1526.

Silene vulgaris (Moench) Garcke has evolved

populations with extremely high levels of copper tolerance. To evaluate the role of metallothioneins (MTs) in copper tolerance in S. vulgaris, we screened a cDNA library derived from a highly copper-tolerant population using Arabidopsis-based MT probes and identified an MT2b-like gene. When expressed in yeast, this gene, SvMT2b, restored cadmium and copper tolerance in different hypersensitive strains. Northern-blot analysis and quantitative reverse transcriptase-PCR showed that plants from the copper-tolerant S. vulgaris populations had significantly higher transcript levels of SvMT2b than plants from the copper-sensitive populations, both in roots and shoots and with and without copper exposure. Southern-blot analysis suggested that the higher expression of the latter allele was caused by gene amplification. Segregating families of crosses between copper-sensitive and copper-tolerant plants exhibited a 1 to 3 segregation for SvMT2b expression. Allele-specific PCR showed that low-expression F(3) plants were homozygous for the allele inherited from the copper-sensitive parent, whereas high-expression plants possessed at least one allele from the tolerant parent. SvMT2b expression did not cosegregate with copper tolerance in crosses between sensitive and tolerant plants. However, a significant cosegregation with copper tolerance did occur in families derived from crosses between moderately tolerant F(3) plants with different SvMT2b genotypes. Thus, overexpression of SvMT2b conferred copper tolerance although only within the genetic background of a copper tolerant plant.

Vandeghinste, N., et al. (2000). "Metallothionein isoform gene expression in zinc-treated human peripheral blood lymphocytes." Cell Mol Biol (Noisy-le-grand) 46(2): 419-433.

Zinc plays an important role in the maintenance of the immune system. While the mechanisms of zinc ions interaction with immune cells are still poorly understood, a striking concurrent effect of zinc is the induction of the biosynthesis of metallothioneins (MT), a group of low molecular weight, cysteine-rich metal-binding proteins, believed to play a role in zinc homeostasis. In humans, they are encoded by a family of genes, located at 16q13 containing 10 functional and 7 non-functional MT isoforms. In this work we analyzed the spectrum of different isoforms in human peripheral blood lymphocytes. It was demonstrated by RT-PCR that the MT-2a, MT-1a, MT-1e, MT-1f, MT-1g, MT-1h and MT-1x genes are expressed in these cells and that these isoforms are further upregulated by zinc, as examined by quantitative RT-PCR. Surprisingly, RT-PCR also showed the presence, even in unstimulated cells, of MT-3 transcripts, which are considered as brain-specific isoforms. In an effort to determine whether MTmRNA abundance is translated into MT protein, MT isolated from zinc-treated lymphocytes by gel chromatography was resolved into 7 metal-binding fractions by using RP-HPLC. Automatic Edman-degradation of the different fractions revealed the presence of MT-2a, MT-1a, MT-1e, MT-1f, MT-1g, MT-1h, MT-1x and MT-1k, an isoform which until now was only identified at the level of protein in human liver and kidney tissue.

Vandier, D., et al. (2000). "Transactivation of the metallothionein promoter in cisplatin-resistant cancer cells: a specific gene therapy strategy." J Natl Cancer Inst 92(8): 642-647.

BACKGROUND: Cisplatin (cis-diamminedichloroplatinum) is one of the most active agents against a broad range of malignancies, including ovarian cancer. Cisplatin resistance appears to be associated with several molecular alterations, including overexpression of metallothionein, a metal-binding protein. In the present study, we attempted to take advantage of metallothionein overexpression to overcome cisplatin resistance. METHODS: Using a virus-free system (liposomes), we sought to express the suicide gene, thymidine kinase (TK), driven by the promoter of the human metallothionein IIa (hMTIIa) gene using the pMT-TK plasmid. We used cisplatin-resistant human ovarian carcinoma cells as a model. RESULTS: We first analyzed metallothionein expression using a ribonuclease protection assay. In comparison to parental cells, the cisplatin-resistant cells were found to have increased expression of metallothionein messenger RNA (mRNA). Metallothionein overexpression in these cells was not associated with an increased copy number of the hMTIIa gene or with different transfection efficiencies. Furthermore, we showed by reverse transcription-polymerase chain reaction analysis that transfection of the pMT-TK plasmid results in a 56-fold higher expression of thymidine kinase mRNA in cisplatin-resistant cells compared with parental cells, consistent with increased metallothionein promoter-mediated transactivation in the cisplatin-resistant cells. Transfection of resistant cells with pMT-TK or a control plasmid (pCD3-TK) resulted in a marked sensitization to ganciclovir, with a 50% cell growth-inhibitory concentration (IC(50)) of 20 microg/mL and 9 microg/mL, respectively. Transfections of the cisplatin-sensitive cells resulted in no sensitization to ganciclovir with pMT-TK (IC(50) 200 microg/mL) and a high sensitization with pCD3-TK (IC(50) = 6 microg/mL). CONCLUSION: These studies suggest that pMT-TK gene therapy may provide an alternative treatment for cisplatin-refractory ovarian tumors.

Varshney, U., et al. (1984). "A frequent restriction fragment length polymorphism in the human metallothionein-II processed gene region is evolutionarily conserved." Mol Biol Med 2(3): 193-206.

Genomic blot analysis of human DNA indicated that metallothioneins are represented by a multi-gene family. Clones containing metallothionein sequences have been isolated and two of these have been identified as metallothionein-I and metallothionein-II processed genes by sequence analysis. The metallothionein-II processed gene in humans shows two restriction fragment length polymorphisms of 4.5 and 4.8 kb (10(3) bases) when EcoRI-digested genomic DNA from various individuals was analysed by Southern blotting. All the three genotypes are found at a high frequency and thus the metallothionein-II processed gene represents a true polymorphic marker. Familial studies also indicate that these restriction fragment length polymorphisms follow the classical Mendelian inheritance. Detailed Southern blot analyses show that this restriction fragment length polymorphism is due to a restriction site polymorphism and is localized at the 5'-flanking region of the metallothionein-II processed gene. Sequence analysis of the suspected region in the 4.8 kb fragment shows that the sequence G*GATTC, which is found 371 nucleotides downstream from the EcoRI site on the 5' end of the 4.8 kb fragment, makes a HinfI site. A transition of *G to A in this sequence in the 4.5 kb allele has resulted in loss of the HinfI site and created an EcoRI site. Thus, this mutation has given rise to this restriction fragment length polymorphism.

Varshney, U., et al. (1986). "Structure, organization, and regulation of human metallothionein IF gene: differential and cell-type-specific expression in response to heavy metals and glucocorticoids." Mol Cell Biol 6(1): 26-37.

We describe a human genomic clone containing the metallothionein (MT) IF and MT IG genes. Southern blot analysis and partial DNA sequence determinations show that these genes are organized in a head-to-head fashion and are located approximately 7.0 kilobases apart from each other. Sequence analysis shows that the MT IF gene contains three exons separated by two introns. All of the intron-exon junctions are defined by the GT-AG rule. The 5' flanking region shows the presence of a duplicated metal regulatory element (TGCGC CCGGCCC) important in heavy-metal induction of this gene and a sequence for its basal level expression (GCGGGGCGGGTGCAAAG). The 5' flanking region is also highly G + C rich (approximately 75%) and contains several GC boxes (GGGCGG), probably important in the binding of transcription factors. The TATAA box and the AATAAA sequence are represented by their variants, the TATCAA box and the AATTAA sequence, respectively. This gene is functional and inducible by heavy metals but not by dexamethasone in mouse LMTK- cells after its transfer on a plasmid containing the herpes simplex virus thymidine kinase gene. Further studies on various human cell lines show that this gene is not expressed in a splenic lymphoblastoid cell line (WI-L2) but is expressed in two hepatoma cell lines (Hep 3B2 and Hep G2) in response to cadmium, zinc, and copper. Dexamethasone appears to have no significant effect on its expression. The studies suggest that the MT IF gene shows cell-type-specific expression and is differentially regulated by heavy metals and glucocorticoids.

Vazquez-Euan, R., et al. (2017). "Partial Gene Sequencing of CYP1A, Vitellogenin, and Metallothionein in Mosquitofish Gambusia yucatana and Gambusia sexradiata." Bull Environ Contam Toxicol 98(1): 41-45.

Ground characteristics in the Yucatan Peninsula make recovery and treatment of wastewater very expensive. This situation has contributed to an increase of pollutants in the aquifer. Unfortunately, studies related to the effects of those pollutants in native organisms are scarce. The aim of this work was to obtain partial sequences of widely known genes used as biomarkers of pollutant effect in Gambusia yucatana and Gambusia sexradiata. The studied genes were: cytochrome P450 1A (CYP1A); vitellogenin (VTG); metallothionein (MT), and two housekeeping genes, 18S and beta-actin. From reported sequences of Gambusia affinis, primers were designed and amplification was done in the local Gambusia species exposed for 48 h to gasoline (100 microL/L, stirred for 24 h pre-exposure). Preliminary results revealed partial sequences of all genes with an approximate average length of 200 bp. BLAST analysis of found sequences indicated a minimum of 97% identity with reported sequences for G. affinis or Gambusia holbrooki showing great similarity.

Waalkes, M. P., et al. (1988). "Increased metallothionein gene expression in 5-aza-2'-deoxycytidine-induced resistance to cadmium cytotoxicity." Chem Biol Interact 66(3-4): 189-204.

The pyrimidine analog, 5-azacytidine (AZA-CR), has been shown to increase the expression of the metallothionein (MT) gene and to induce tolerance to cadmium toxicity. Since incorporation into DNA of AZA-CR appears to be required for this effect, the deoxynucleoside of AZA-CR should also be effective. Therefore, this study was undertaken to assess the effect of 5-aza-2'-deoxycytidine (AZA-CdR) pretreatment on cadmium-induced cytotoxicity and MT expression in cultured cells. TRL 1215 cells in log phase of growth were exposed to AZA-CdR (0.4, 0.8, 4.0, 8.0 microM) followed 48 h later by the addition of cadmium (10 microM). MT concentrations were measured 24 h after the addition of cadmium. AZA-CdR alone caused modest, dose-related increases in MT levels (2.3-fold maximum), while cadmium alone resulted in a 9.5-fold increase. Pretreatment with AZA-

CdR in combination with cadmium caused a 19--24-fold increase in cellular MT at all doses of AZA-CdR. Addition of the DNA synthesis inhibitor, hydroxyurea (HU), to the incubation medium during AZA-CdR exposure prevented the enhancing effect of the analog on cadmium induction of MT accumulation. Time course studies revealed that AZA-CdR pretreatment reduced the time required for cadmium to induce MT levels from 4--8 h to 0--2 h. AZA-CdR pretreated cells placed in suspension with cadmium (125 microM) showed a marked reduction in cadmium-induced cytotoxicity as reflected by reduced glutamic-oxaloacetic transaminase (GOT) loss. Uptake studies showed that AZA-CdR pretreatment had no effect on cadmium transport during the initial phases of exposure, indicating that an alteration in the toxicokinetics of the metal did not account for the reduction in toxicity. AZA-CdR did, however, cause hypomethylation of the MT-I gene. These results suggest that AZA-CdR pretreatment induces tolerance to cadmium toxicity by increasing the genetic expression of MT possibly through hypomethylation of the MT gene.

Wahid, M., et al. (2017). "Cadmium accumulation and metallothionein gene expression in the liver of swamp eel (Monopterus albus) collected from the Mae Sot District, Tak Province, Thailand." Genet Mol Res 16(3).

Cadmium (Cd) is produced mainly as a by-product of zinc mining. In Thailand, the largest zinc mine is located in the Mae Sot district, Tak Province. Samples of Monopterus albus were collected from paddy fields in 4 sites, three downstream and one upstream from the zinc mine. The upstream site was considered to be uncontaminated while the three downstream sites were considered to be contaminated with Cd. Studies on the accumulation level of cadmium were conducted on the liver of the fish using the atomic absorption spectrophotometer technique. The metallothionein (MT) gene expression level in the liver, as a potential biomarker for long-term Cd exposure in their natural habitat, was also assessed. The level of hepatic MT gene expression was performed by quantitative real-time PCR. The result showed that Cd accumulation in the liver was much higher in swamp eels collected from the downstream sites when compared to those collected from the upstream site. The hepatic MT level in the upstream site was 0.75-fold, while the other three downstream sites were 0.36-, 4.44- and 0.94-fold. There is no parallel correlation between hepatic cadmium levels and hepatic MT gene expression. This study then suggests that MT gene expression biomarkers might be not suitable for swamp eels with prolonged exposure to Cd.

Wallace, S. M., et al. (1996). "Effect of zinc on metallothionein content of CHO cells transfected with metallothionein gene under the control of a non-inducible promoter." Biochem Soc Trans 24(2): 237S.

Wan, G., et al. (2009). "Differential regulation of zebrafish metallothionein-II (zMT-II) gene transcription in ZFL and SJD cell lines by metal ions." Aquat Toxicol 91(1): 33-43.

Two alleles of a zebrafish metallothionein II gene (zMT-II) promoter (zMT-IIA and zMT-IIB) containing 10 MREs in the 5'-flanking region (1514bp) were identified in zebrafish. These putative MREs were confirmed via electrophoretic mobility shift assay (EMSA) to have binding activities from the cellular and nuclear extracts of a zebrafish cell line, ZFL. Transient gene expression studies using zebrafish liver (ZFL) and caudal fin (SJD) cell lines also confirmed that the most distal cluster of MREs contributed to the maximal induction of zMT-IIA activity by Zn(2+) and that this Zn(2+) induction was dose-dependent. Further transient gene expression assay of the zMT-IIA gene promoter was carried out to study the effects of various metal ions (Zn(2+), Cd(2+), Cu(2+), Hg(+), As(3+), As(5+), Cr(3+) and Cr(6+)), and Zn(2+) and Cd(2+) were found to be the most efficient MT gene inducers of zMT-II. As(3+) was a weak inducer of zMT-II in the two cell lines, and Hg(+) caused significant induction only in the SJD cells. No significant induction was found in the other metal ion exposures. EMSA also identified transcription factor(s) of two different sizes from the cytoplasmic and nuclear extracts of the ZFL cells that were able to bind with the MREs, but no increase in MRE binding was detected in the extracts of these cells after Zn(2+) or Cd(2+) treatment, compared with untreated control cells. The mechanisms of MT gene transcription induction via metal ions are discussed herein.

Wan, M., et al. (1995). "Regulation of metallothionein gene expression in Cd- or Zn-adapted RK-13 cells." Experientia 51(6): 606-611.

We explored the molecular genetics underlying the massive induction of isoMTs by Zn2+ or Cd2+ in metal tolerant rabbit kidney (RK-13) sub-line cells, using band shift assays and Southern blotting analysis. In sub-line cells accommodated to intermediate metal concentrations (100 microM Zn2+; 1-20 microM Cd2+) evidence suggested that the increase in the capacity for isoMT synthesis is brought about by an increased binding activity of the nuclear transcription factors MTF-1 and Sp1. Using quantitative band shift analysis with a mouse MRE-d oligonucleotide probe, the binding of both transcription factors was found to be enhanced two to three times over the binding activity measured in the unexposed parental RK-13 cells. Their increase in binding activity is probably the cause of the overexpression of MT genes and the development of metal tolerance in these cells. In cells tolerant to the highest concentrations of metal the analysis of Southern blot signals revealed MT gene amplification to be the most probable cause of the

increased MT production. Thus, in cells of sub-lines growing in the presence of 350 microM Zn2+, two of the isoMT genes were coordinately triplicated and in cells tolerant to 150 microM Cd2+ one isoMT gene was amplified two-fold.

Wang, B., et al. (2008). "PCR arrays identify metallothionein-3 as a highly hypoxia-inducible gene in human adipocytes." Biochem Biophys Res Commun 368(1): 88-93.

Hypoxia-signalling pathway PCR arrays were used to examine the integrated response of human adipocytes to low O(2) tension. Incubation of adipocytes in 1% O(2) for 24h resulted in no change in the expression of 63 of the 84 genes on the arrays, a reduction in expression of 9 genes (including uncoupling protein 2) and increased expression of 12 genes. Substantial increases (>10-fold) in leptin, angiopoietin-like protein 4, VEGF and GLUT-1 mRNA levels were observed. The expression of one gene, metallothionein-3 (MT-3), was dramatically (>600-fold) and rapidly (by 60 min) increased by hypoxia. MT-3 gene expression was also substantially induced by hypoxia mimetics (CoCl(2), desferrioxamine, dimethyloxalylglycine), indicating transcriptional regulation through HIF-1. Hypoxia additionally induced MT-3 expression in preadipocytes, and MT-3 mRNA was detected in human (obese) subcutaneous and omental adipose tissue. MT-3 is a highly hypoxia-inducible gene in human adipocytes; the protein may protect adipocytes from hypoxic damage.

Wang, J., et al. (2020). "Quantitative assessment of the association of polymorphisms in the metallothionein 2A gene with cancer risk." J Int Med Res 48(8): 300060520947937.

OBJECTIVE: The aim of the study was to quantitatively assess the association of metallothionein 2A (MT2A) polymorphisms rs28366003 and rs1610216 with cancer risk. METHODS: Crude odd ratios (OR) with 95% confidence intervals (CI) were used to estimate associations of the polymorphisms with cancer risk. RESULTS: Six eligible case-control studies with 1899 cases and 2437 controls focused on rs28366003, and three of those six studies, with 548 cases and 926 controls, additionally focused on rs1610216. Pooled analysis showed that MT2A rs28366003 and rs1610216 were associated with cancer risk: (AG + GG) vs. AA, OR = 2.67; GG vs. (AG + AA), OR = 5.97; GG vs. AA, OR = 6.80; AG vs. AA, OR = 2.46; G vs. A, OR = 2.67 for rs28366003; and CC vs. (TC+TT), OR = 2.51; CC vs. TT, OR = 2.42 for rs1610216. Subgroup analysis based on ethnicity showed a significant association of rs28366003 with cancer risk in Asian and Caucasian populations. However, a significant association of rs1610216 with cancer risk was found only in the Asian population. CONCLUSION: MT2A rs28366003 and rs1610216 polymorphisms were associated with cancer risk and might serve as genetic biomarkers for predicting cancer risk. However, larger studies are needed to confirm these findings.

Wang, S. H., et al. (1997). "Cloning of porcine neuron growth inhibitory factor (metallothionein III) cDNA and expression of the gene in Saccharomyces cerevisiae." Gene 203(2): 189-197.

Growth inhibitory factor (GIF), a member of the metallothionein (MT) family, is also known as MTIII. This protein distinguishes itself from other MT isoforms by exerting an inhibitory effect on cortical neuron growth instead of metal ion buffering. In this work, we cloned MTIII genes from a porcine brain cDNA library. Two species of clones were isolated that vary with respect to one nt in the coding sequence. This discrepancy results in the translation of two MTIII primary structures having a different amino acid at residue 46. Herein, both MTIII cDNAs were constructed into an expression vector and transformed into yeast cells, respectively. The yeast carrying either MTIII gene displayed a similar metal tolerance when cultured in a medium containing metal. The resistance to metal toxicity was attributed to the expression of MTIII gene which was confirmed by RNA and protein analyses. The characteristics of the protein stability, metal binding affinity and ultraviolet absorption spectrum of the yeast produced MTIII are also compared with those of MTII. The comparison reveals that both MTs have similar physical characteristics. Moreover, circular dichroism spectrum of Cd saturated MTIII was analyzed as well. Typical Cys-Cd bands for MTII appear in the spectrum, indicating similar metal-thiol interactions for MTIII to those for other MT isoforms.

Wang, Z. C., et al. (2019). "Effect of dietary zinc pectin oligosaccharides chelate on growth performance, enzyme activities, Zn accumulation, metallothionein concentration, and gene expression of Zn transporters in broiler chickens1." J Anim Sci 97(5): 2114-2124.

This study was to investigate the effect of zinc pectin oligosaccharides chelate (Zn-POS) on growth performance, serum enzyme activities, tissue zinc accumulation, metallothionein (MT) concentrations, and gene expression of zinc transporters (ZnT) in broilers. Five hundred forty 1-d-old Arbor Acres broiler chicks were randomly assigned to 5 dietary groups with 6 replicates of 18 birds per replicate. The diets were formulated with the same supplemental Zn level (80 mg/kg diet) but different amount of the Zn-POS: 0, 200, 400, 600, and 800 mg Zn-POS/kg diet. ZnSO4 was used to adjust to the desired amount of the Zn (80 mg/kg) in the Zn-POS diets. Broilers were fed with the experimental diets for 42 d including the starter (days 1 to 21) and grower (days 22 to 42) phases. Our results showed that dietary supplementation of Zn-POS

linearly and quadratically increased ($P < 0.05$) the average daily gain and gain-to-feed ratio during 22 to 42 d and 1 to 42 d as well as body weight on day 42, whereas reduced ($P < 0.05$) the sum of mortality and lag abnormalities in broilers on day 42. Besides, serum alkaline phosphatase and copper-zinc superoxide dismutase activities increased ($P < 0.05$) linearly and quadratically in response to dietary Zn-POS supplemental level on day 42. Dietary Zn-POS supplementation increased Zn accumulation in serum (linear, $P < 0.05$), liver (linear, $P < 0.05$), and pancreas (linear and quadratic, $P < 0.05$). In addition, Zn-POS supplementation linearly and quadratically increased ($P < 0.01$, $P < 0.05$, respectively) MT concentrations in liver and pancreas of broilers. Pancreatic mRNA levels of MT, ZnT-1, and ZnT-2 increased ($P < 0.05$) linearly and quadratically, and the mRNA expression of metal response element-binding transcription factor-1 increased linearly ($P < 0.05$), in response to dietary Zn-POS supplementation. In conclusion, supplementation of Zn-POS in the diet increases Zn enrichment in the metabolic organs such as liver and pancreas and promotes productive performance in broilers.

Wassermann, K., et al. (1992). "Overexpression of metallothionein in Chinese hamster ovary cells and its effect on nitrogen mustard-induced cytotoxicity: role of gene-specific damage and repair." Cancer Res 52(24): 6853-6859.

Overexpression of metallothionein in mammalian cells has been associated with protection from cytotoxic chemicals and acquired resistance of tumors to cytotoxic drugs. The mechanism of this effect, however, remains unclear. We have explored whether cytotoxicity of the bifunctional alkylating agent nitrogen mustard was correlated with the extent of DNA damage formation and repair in the metallothionein gene regions in Chinese hamster ovary cells. The DNA damage and repair were examined in metallothionein-overexpressing, cadmium-resistant Chinese hamster ovary cells, Cdr200T1, with or without zinc-induced transcriptional activation, and in the parental CHO-met- cell line. The zinc-induced Cdr200T1 cells tolerated significantly higher doses of nitrogen mustard than did the uninduced Cdr200T1 variant. The parental CHO-met- cells, which did not have any detectable metallothionein expression, were even more resistant to nitrogen mustard than the zinc-induced Cdr variants. Nitrogen mustard-induced N-alkylpurines were formed with a higher frequency in inactive genomic regions than in the active genes. The removal of N-alkylpurines was similar in the active MT I gene region in Cdr200T1 and the silent MT I gene region in the parental cells, and the expression of these genes was determined by Northern assay. The MT II gene-containing region was repaired less efficiently than the MT I gene, independently of zinc induction. Further, preferential repair of nitrogen mustard-induced N-alkylpurines were detected in a single copy of the essential active dihydrofolate reductase gene as compared to a downstream noncoding region. This preferential repair was unaffected by the presence of zinc. Neither damage formation nor repair kinetics in the MT gene regions seemed to parallel the observed spectrum of sensitivity to HN2.

Webb, M. L., et al. (1987). "Novobiocin inhibits initiation of RNA polymerase II-directed transcription of the mouse metallothionein-I gene independent of its effect on DNA topoisomerase II." Nucleic Acids Res 15(20): 8547-8560.

The requirement for ATP hydrolysis in the initiation of RNA polymerase II (Pol II)-directed transcription and the relationship between ATP and novobiocin action led us to investigate whether novobiocin could inhibit transcription of the mouse metallothionein-I (MT-I) gene. Novobiocin inhibited the MT-I gene transcription in a fractionated rat hepatoma nuclear extract in a dose-dependent manner by direct interaction with a nuclear factor(s). This interaction prevented formation of stable preinitiation complexes but did not affect elongation of MT-I mRNA. Preincubation of the nuclear extract with ATP prevented the action of novobiocin on MT-I gene transcription. Although novobiocin is known to inhibit DNA topoisomerase II, VM-26, a specific inhibitor of this enzyme had no effect on the transcription. These results indicate that novobiocin blocks the Pol II-directed transcription by inhibiting formation of preinitiation complexes at an ATP-dependent step.

Weis, J. H. and J. G. Seidman (1985). "The expression of major histocompatibility antigens under metallothionein gene promoter control." J Immunol 134(3): 1999-2003.

A model system has been developed that provides insights into the mechanisms that control the amount of H-2 antigen on the cell surface. Hybrid genes have been constructed by using the metallothionein gene promoter to replace the H-2 gene promoter. The hybrid genes have been introduced into murine L cells and their expression has been studied. Cells containing the hybrid genes contain 20- to 60-fold more H-2 mRNA than nontransfected L cells, since the metallothionein gene promoter is much more active than the H-2 promoter. However, the total amount of H-2 antigen on the surface of the transfected L cell is similar to the amount of H-2 antigen on the normal L cell. Even after transcription from the metallothionein promoter is induced by the addition of cadmium to the cell culture medium, the amount of H-2 antigen on the surface of cells containing the hybrid genes does not increase. We conclude that the amount of H-2 antigen is controlled by events that occur after

gene transcription. Evidence is presented that suggests that these post-transcriptional mechanisms may cause the expression of threefold more H-2Dd than H-2Ld on the surface of BALB/c cells. Furthermore, we suggest that the 5' untranslated portion of the H-2 mRNA is not important for directing the growing H-2 polypeptide to the cell surface.

Welch, J., et al. (1989). "The CUP2 gene product regulates the expression of the CUP1 gene, coding for yeast metallothionein." EMBO J 8(1): 255-260.

The yeast CUP1 gene codes for a copper-binding protein similar to metallothionein. Copper sensitive cup1s strains contain a single copy of the CUP1 locus. Resistant strains (CUP1r) carry 12 or more multiple tandem copies. We isolated 12 ethyl methane sulfonate-induced copper sensitive mutants in a wild-type CUP1r parental strain, X2180-1A. Most mutants reduce the copper resistance phenotype only slightly. However, the mutant cup2 lowers resistance by nearly two orders of magnitude. We cloned CUP2 by molecular complementation. The smallest subcloned fragment conferring function was approximately 2.1 kb. We show that CUP2, which is on chromosome VII, codes for or controls the synthesis or activity of a protein which binds the upstream control region of the CUP1 gene on chromosome VIII. Mutant cup2 cells produced extremely low levels of CUP1-specific mRNA, with or without added copper ions and lacked a factor which binds to the CUP1 promoter. Integrated at the cup2 site, the CUP2 plasmid restored the basal level and inducibility of CUP1 expression and led to reappearance of the CUP1-promoter binding factor. Taken collectively, our data establish CUP2 as a regulatory gene for expression of the CUP1 metallothionein gene product.

Westin, G. and W. Schaffner (1988). "A zinc-responsive factor interacts with a metal-regulated enhancer element (MRE) of the mouse metallothionein-I gene." EMBO J 7(12): 3763-3770.

Heavy metal ions are effective inducers of metallothionein gene transcription. The metal response is dependent on short DNA motifs, so-called MREs (metal responsive elements) that occur in multiple copies in the promoter region of these genes. We have analysed an MRE of the mouse metallothionein-I gene (MREd) and we demonstrate that this can function over long distances as a bona fide metal ion-inducible enhancer. The transcription factor Sp1 and a zinc-inducible factor, designated MTF-1, bind to the MREd enhancer in vitro. The combined use of MREd mutants in a transient assay in HeLa cells and a competition band shift assay show that the zinc-inducible formation of the MTF-1/DNA complex in vitro correlates with zinc-inducible transcription in vivo. A chemical methylation interference assay revealed remarkably similar but non-identical guanine interference patterns for the MTF-1 and Sp1 complexes, which may mean that MTF-1 is related to the Sp1 factor.

Winge, D. R., et al. (1994). "The metallothionein structural motif in gene expression." Adv Inorg Biochem 10: 1-48.

Metalloregulation in eukaryotic organisms is poorly understood. Only a limited number of physiological processes are currently known to be regulated by metal ions. Copper salts stimulate transcription of MT and SOD genes in fungi and repress expression of cytochrome c6 in algae. The Cu-activation of gene expression in fungi is mediated by a Cu1+ specific sensor protein. The mechanism of Cu-activation of the sensor molecule, ACE1 (and probably AMT1), appears to be the formation of a CuS polymetallic cluster as the structural core of the proteins. Structural similarities between ACE1, AMT1 and metallothionein suggest that the MT motif is a good structural model to explain the metal-specific activation and specificity of the two signal transducing proteins. Metal ion specificity is achieved by the propensity of proteins with a MT motif to form multinuclear CuS centers. Coordination inorganic chemistry appears to be the driving force for Cu1+ metalloregulation in biology. The metalloregulation of transcriptional activation of mammalian MT genes will be intriguing as metal ion selectivity is not as apparent. The MT motif is not expected to be a highly redundant structural theme. The intriguing observation by Uchida et al. (154) that the growth inhibitory factor (MTIII) deficient in the brains of Alzheimer's patients is homologous to metallothioneins raises the likelihood that coordination chemistry will be critical in stabilizing the bioactive form of MTIII. The motif may be observed in yet to be identified metalloregulatory proteins that regulate other processes in a Cu- or Zn-specific manner. Formation of metal:thiolate polymetallic clusters allows a significant volume of the protein structure to be altered, so metal-induced structural dynamics are possible. CuS polynuclear clusters may be more general than the MT motif in biology. The only molecules currently known to form CuS polynuclear clusters include MT, ACE1 and the Cu(gamma EC)nG complexes, although AMT1 and MTIII are expected to be the next members in the list. Three other Cys-rich proteins, papilloma viral E7 and LIM motif-containing CRP and CRIP, isolated as Zn2+ proteins exhibit facile metal exchange in vitro with Cu1+. The resulting Cu1+ proteins show optical properties similar to CuMTs (202,203). Although CuS clusters may form in E7 and LIM-containing proteins, it is premature to ascribe any biological significance to the Cu1+ conformers.

Won, E. J., et al. (2011). "Molecular cloning and expression of novel metallothionein (MT) gene in the polychaete Perinereis nuntia exposed to metals."

Environ Sci Pollut Res Int 19(7): 2606-2618.

To report a novel metallothionein (MT) gene and evaluate its potency as a biomarker, we clone this MT gene and measured the expression levels in the metal-exposed polychaete Perinereis nuntia. Accumulated metal contents and metallothionein-like proteins (MTLPs), which have been recognized as potential biomarkers, were compared with the relative mRNA expressions of the MT gene of P. nuntia (Pn-MT). In addition, the metal-binding affinity was estimated by recombinant Pn-MT protein. Pn-MT having high cysteine residues with three metal response elements in the promoter region closely clusters with those of other invertebrates. The accumulation patterns of metals were dependent on the exposure times in lead (Pb), cadmium (Cd), and copper (Cu) exposure. Particularly, both MTLP levels and relative mRNA expressions of MT were increased with accumulated metal contents and exposure time in P. nuntia exposed to Pb and Cd. There was no significant modulation of the Pn-MT gene in polychaetes exposed to Zn and As. However, the metal-binding ability of the recombinant Pn-MT protein provides a clear evidence for a high affinity of MT to several metal elements. These results suggest that Pn-MT would play an important role in the detoxification and/or sequestration of specific metals (e.g., Pb and Cd) in P. nuntia and have potential as a molecular biomarker in the monitoring of the marine environment using a polychaete.

Woo, S., et al. (2006). "Heavy metal-induced differential gene expression of metallothionein in Javanese medaka, Oryzias javanicus." Mar Biotechnol (NY) 8(6): 654-662.

A metallothionein (MT) gene was isolated for the first time from Javanese medaka, Oryzias javanicus, which shows high adaptability from freshwater to seawater. The full-length cDNA of MT from O. javanicus (OjaMT) comprises 349 bp, excluding the poly(A)+ stretch, and codes for a total of 60 amino acids. The positions of cysteine residues are highly conserved. The pattern of OjaMT expression induced by six heavy metals was analyzed via real-time quantitative polymerase chain reaction (PCR). The level of hepatic OjaMT mRNA was increased in a dose-dependent manner by Ag, Cd, Cu, and Zn after 24 h of exposure. However, after Cr and Ni exposure, a significant decrease in OjaMT levels was observed. Cadmium-induced OjaMT expression was detectable in fishes as young as 3 months. After Cd exposure, OjaMT induction was prominent in intestine and liver and moderate in muscle and gill. OjaMT mRNA levels could represent a good biomarker for monitoring heavy metals in seawater.

Wood, D. P., Jr., et al. (1993). "Metallothionein gene expression in bladder cancer exposed to cisplatin." Mod Pathol 6(1): 33-35.

Some 68% of bladder tumors will respond to cisplatin-based chemotherapy but only 30% will have a durable response. Recent studies have suggested that the metallothionein (MT) gene may produce cisplatin drug resistance in cell lines. To determine the role of MT gene overexpression in human tumors resistant to cisplatin, we evaluated 19 bladder tumors, seven of which had been exposed to cisplatin, for MT mRNA expression. By Northern analysis, four of the seven tumors exposed to cisplatin overexpressed the MT gene compared to untreated tumors. Of the three treated tumors without MT overexpression, one was a relapse 4 yr after the last dose of cisplatin and the other two received only one dose of chemotherapy. MT gene overexpression was found in some tumors that had failed cisplatin chemotherapy and may be a mechanism for drug resistance in bladder cancer.

Wooten, D. C., et al. (2016). "A plasmid containing the human metallothionein II gene can function as an antibody-assisted electrophoretic biosensor for heavy metals." J Immunotoxicol 13(1): 55-63.

Different forms of heavy metals affect biochemical systems in characteristic ways that cannot be detected with typical metal analysis methods like atomic absorption spectrometry. Further, using living systems to analyze interaction of heavy metals with biochemical systems can be laborious and unreliable. To generate a reliable easy-to-use biologically-based biosensor system, the entire human metallothionein-II (MT-II) gene was incorporated into a plasmid (pUC57-MT) easily replicated in Escherichia coli. In this system, a commercial polyclonal antibody raised against human metal-responsive transcription factor-1 protein (MTF-1 protein) could modify the electrophoretic migration patterns (i.e. cause specific decreases in agarose gel electrophoretic mobility) of the plasmid in the presence or absence of heavy metals other than zinc (Zn). In the study here, heavy metals, MTF-1 protein, and polyclonal anti-MTF-1 antibody were used to assess pUC57-MT plasmid antibody-assisted electrophoretic mobility. Anti-MTF-1 antibody bound both MTF-1 protein and pUC57-MT plasmid in a non-competitive fashion such that it could be used to differentiate specific heavy metal binding. The results showed that antibody-inhibited plasmid migration was heavy metal level-dependent. Zinc caused a unique mobility shift pattern opposite to that of other metals tested, i.e. Zn blocked the antibody ability to inhibit plasmid migration, despite a greatly increased affinity for DNA by the antibody when Zn was present. The Zn effect was reversed/modified by adding MTF-1 protein. Additionally, antibody inhibition of plasmid mobility was resistant to heat pre-treatment and trypsinization, indicating absence of residual DNA extraction-resistant bacterial DNA binding proteins. DNA binding by anti-DNA antibodies may be commonly enhanced by

xenobiotic heavy metals and elevated levels of Zn, thus making them potentially effective tools for assessment of heavy metal bioavailability in aqueous solutions and fluid obtained from metal implant sites.

Wright, C. F., et al. (1988). "Autoregulation of the yeast copper metallothionein gene depends on metal binding." J Biol Chem 263(3): 1570-1574.

The yeast CUP1 gene product, copper metallothionein, acts to repress the basal transcription of its own structural gene. By creating a series of truncation and amino acid substitutions in CUP1, we show that the ability of the protein to autoregulate is directly correlated to its ability to bind and detoxify copper. These results support a model in which metallothionein controls the level of free intracellular copper available to interact with positive transcription factors. In addition, mutations in chemically equivalent cysteine residues were functionally dissimilar, suggesting that partial sites in the molecule are critical for the formation of the sulfur-metal cluster.

Wu, C. C. and A. M. Fallon (1997). "Evaluation of a heterologous metallothionein gene promoter in transfected mosquito cells." Comp Biochem Physiol B Biochem Mol Biol 116(3): 353-358.

Mosquito cells from the C7-10 Aedes albopictus line were transfected with a recombinant plasmid containing the Escherichia coli galactokinase gene under control of the promoter from the Drosophila melanogaster metallothionein gene, Mtn. Consistent with what has been observed with heterologous metallothionein promoters in several vertebrate systems, treatment of transiently transfected mosquito cells with CuSO4 or CdCl2 induced a 2- to 5-fold increase in galactokinase gene expression. Levels of enzyme activity were not increased in tests using stably transformed lines despite wide ranges in the number of transfected gene copies detected in Southern blots. The importance of comparative studies with gene constructs that may eventually be used to produce genetically modified mosquitoes is underscored by the apparent variability in activity of heterologous promoters from D. melanogaster in different mosquito cell lines.

Wu, C. S., et al. (2014). "The promoter and the 5'-untranslated region of rice metallothionein OsMT2b gene are capable of directing high-level gene expression in germinated rice embryos." Plant Cell Rep 33(5): 793-806.

KEY MESSAGE: Critical regions within the rice metallothionein OsMT2b gene promoter are identified and the 5'-untranslated region (5'-UTR) is found essential for the high-level promoter activity in germinated transgenic rice embryos. Many metallothionein (MT) genes are highly expressed in plant tissues. A rice subfamily p2 (type 2) MT gene, OsMT2b, has been shown previously to exhibit the most abundant gene expression in young rice seedling. In the present study, transient expression assays and a transgenic approach were employed to characterize the expression of the OsMT2b gene in rice. We found that the OsMT2b gene is strongly and differentially expressed in germinated rice embryos during seed germination and seedling development. Histochemical staining analysis of transgenic rice carrying OsMT2b::GUS chimeric gene showed that high-level GUS activity was detected in germinated embryos and at the meristematic part of other tissues during germination. Deletion analysis of the OsMT2b promoter revealed that the 5'-flanking region of the OsMT2b between nucleotides -351 and -121 relative to the transcriptional initiation site is important for promoter activity in rice embryos, and this region contains the consensus sequences of G box and TA box. Our study demonstrates that the 5'-untranslated region (5'-UTR) of OsMT2b gene is not only necessary for the OsMT2b promoter activity, but also sufficient to augment the activity of a minimal promoter in both transformed cell cultures and germinated transgenic embryos in rice. We also found that addition of the maize Ubi intron 1 significantly enhanced the OsMT2b promoter activity in rice embryos. Our studies reveal that OsMT2b351-ubi(In) promoter can be applied in plant transformation and represents potential for driving high-level production of foreign proteins in transgenic rice.

Wu, H., et al. (2015). "Bioaccumulation, morphological changes, and induction of metallothionein gene expression in the digestive system of the freshwater crab Sinopotamon henanense after exposure to cadmium." Environ Sci Pollut Res Int 22(15): 11585-11594.

To study the responses of digestive system of the freshwater crab Sinopotamon henanense to the exposure with cadmium (Cd), crabs were acutely exposed to 7.25, 14.50, and 29.00 mg/l Cd for 96 h and subchronically exposed to 0.725, 1.450, and 2.900 mg/l for 21 days. Cd bioaccumulation in the hepatopancreas and digestive tract (esophagus and intestine) was examined. Furthermore, histopathological alterations of the esophagus, midgut, hindgut, and hepatopancreas were assessed in animals from the 29.0 and 2.90 mg/l Cd treatment groups, and expression of metallothionein messenger RNA (MT mRNA) in the hepatopancreas and intestine was measured in all treatment groups. The results showed difference in the middle and high concentrations between acute and subchronic treatment groups. Cd content in digestive tract after acute 14.5 and 29.0 mg/l Cd exposure was significantly higher than that at subchronic 1.45 and 2.90 mg/l exposure, but Cd levels in hepatopancreas were not significantly different under the same condition. Acute exposure to Cd induced greater morphological damage than subchronic exposure: large areas of epithelial cells

were necrotic in hepatopancreas and midgut, which detached from the basal lamina. Vacuolated muscle cells were observed in the hindgut of animals from the acute exposure group, but the changes of esophageal morphology were not obvious after acute or subchronic treatments. The expression of MT mRNA increased with increasing Cd concentration, and MT mRNA level in acute exposure groups was significantly lower when compared to the subchronic exposure groups. Higher Cd content and lower MT mRNA expression in the acutely exposed groups may be responsible for more severe damage of digestive system in these exposure groups.

Wu, Y., et al. (2008). "Overlapping gene expression profiles of cell migration and tumor invasion in human bladder cancer identify metallothionein 1E and nicotinamide N-methyltransferase as novel regulators of cell migration." Oncogene 27(52): 6679-6689.

Cell migration is essential to cancer invasion and metastasis and is spatially and temporally integrated through transcriptionally dependent and independent mechanisms. As cell migration is studied in vitro, it is important to identify genes that both drive cell migration and are biologically relevant in promoting invasion and metastasis in patients with cancer. Here, gene expression profiling and a high-throughput cell migration system answers this question in human bladder cancer. In vitro migration rates of 40 microarray-profiled human bladder cancer cell lines were measured by radial migration assay. Genes whose expression was either directly or inversely associated with cell migration rate were identified and subsequently evaluated for their association with cancer stage in 61 patients. This analysis identified genes known to be associated with cell invasion such as versican, and novel ones, including metallothionein 1E (MT1E) and nicotinamide N-methyltransferase (NNMT), whose expression correlated positively with cancer cell migration and tumor stage. Using loss of function analysis, we show that MT1E and NNMT are necessary for cancer cell migration. These studies provide a general approach to identify the clinically relevant genes in cancer cell migration and mechanistically implicate two novel genes in this process in human bladder cancer.

Xie, T., et al. (2004). "Identification and characterization of metallothionein-1 and -2 gene expression in the context of (+/-)3,4-methylenedioxymethamphetamine-induced toxicity to brain dopaminergic neurons." J Neurosci 24(32): 7043-7050.

In mice, the recreational drug (+/-)3,4-methylenedioxymethamphetamine [MDMA ("ecstasy")] produces a selective toxic effect on brain dopamine (DA) neurons. Using cDNA microarray technology in combination with an approach designed to facilitate recognition of relevant changes in gene expression, the present studies sought to identify genes potentially involved in murine MDMA-induced toxicity to DA neurons. Of 15,000 mouse cDNA fragments studied, metallothionein (Mt)-1 and Mt2 emerged as candidate genes possibly involved in MDMA-induced toxicity to DA neurons. Northern blot analysis confirmed the microarray findings and revealed a dynamic upregulation of Mt1 and Mt2 mRNA in the ventral midbrain within 4-12 hr after MDMA treatment. Western blot analysis showed a similar increase in MT protein levels, with peak times occurring subsequent to increases in mRNA levels. Mt1-2 double knock-out mice were more vulnerable to MDMA-induced toxicity to DA neurons than corresponding wild-type mice. Stimulation of endogenous expression of MT protein with zinc acetate conferred complete protection against MDMA-induced toxicity to DA neurons, and administration of exogenous MT protein afforded partial protection. Collectively, these results indicate that MDMA-induced toxicity to DA neurons is associated with increased Mt1 and Mt2 gene transcription and translation, possibly as part of a neuroprotective mechanism. The present findings may have therapeutic implications for neuropathological conditions involving DA neurons.

Xu, C. (1993). "cDNA cloning of a mouse factor that activates transcription from a metal response element of the mouse metallothionein-I gene in yeast." DNA Cell Biol 12(6): 517-525.

A cDNA that encodes a mouse factor that activates expression from a metal response element of the mouse metallothionein-I gene has been isolated by complementation cloning in yeast cells. The cDNA encodes a peptide with a maximum length of 99 amino acids that includes a single zinc finger sequence. In yeast cells, the cloned factor induces transcription from the metal response element in a sequence-specific but metal-independent fashion. The cDNA hybridizes to a 550-base mRNA that is constitutively expressed in mouse tissue culture cells. The ability of the mouse factor to activate transcription in yeast cells is dependent upon the carbon source.

Xu, X., et al. (2013). "Metallothionein gene transfection reverses the phenotype of activated human hepatic stellate cells." J Pharmacol Exp Ther 346(1): 48-53.

Metallothionein (MT) gene therapy leads to resolution of liver fibrosis in mouse model. The present study was undertaken to test the hypothesis that reversal of the phenotype of activated hepatic stellate cells (HSCs) contributes to the fibrinolysis effect of MT. Human HSC LX-2 cells were activated after they were cultured for 24 hours, as indicated by expression of alpha-smooth muscle actin (alpha-SMA) and collagen-I and depressed expression of collagenases.

Transfection with a plasmid containing human MT-IIA gene in the activated HSCs effectively increased the protein level of MT. The expression of MT was accompanied by the reduction in protein levels of alpha-SMA and collagen-I and a decrease in their mRNA levels. Of importance, MT gene transfection resulted in upregulation of matrix metalloproteinases 1, 8, and 13, which are involved in the resolution of liver fibrosis. This study demonstrates that reversal of the phenotype of activated HSCs, particularly the upregulation of collagenases, is likely to be involved in the resolution of liver fibrosis observed in MT gene therapy.

Yajima, H., et al. (1996). "Construction and characterization of a recombinant adenovirus vector carrying the human preproinsulin gene under the control of the metallothionein gene promoter." Biochem Biophys Res Commun 229(3): 778-787.

A new adenovirus vector carrying human-preproinsulin (h-PPI) genomic DNA, which was placed under the control of the mouse metallothionein gene promoter, was constructed. In the recombinant virus-infected cells, h-PPI gene expression increased as a function of ZnSO4 concentration. Reversed-phase high-performance liquid chromatography analysis revealed that the recombinant adenovirus-infected cells secreted immature insulin containing proinsulin and incorrectly processed insulin. Tyrosyl phosphorylation of human insulin receptor substrate 1 occurred when HepG2 cells were treated with the cultured medium, indicating that the h-PPI gene product was functionally active in vitro. We also examined the biological activity of the product using diabetic severe combined immunodeficient mice and confirmed that the h-PPI gene product reduced the blood glucose concentration in vivo. This study suggests that the adenovirus vector can be used to express a foreign gene under the control of an external promoter in various human cells.

Yamada, H., et al. (2004). "Ultraviolet irradiation increases the sensitivity of cultured human skin cells to cadmium probably through the inhibition of metallothionein gene expression." Toxicol Appl Pharmacol 200(3): 251-257.

We previously developed an apparatus that can irradiate cultured cells with monochromatic ultraviolet (UV) rays to exactly assess the biological effects of UV components on mammalian cells. Using this device, we studied the effects of UV in and near the UVB region on the general as well as specific protein synthesis of the human skin-derived NB1RGB cells. We found that Cd-induced synthesis of metallothioneins (MTs), which are the proteins involved in the protection against heavy metals and oxidative stress, is inhibited by UV at 280 nm more extensively than total protein synthesis. Such an inhibition was observed when MTs were induced by different inducers such as Cd, Zn, and dexamethasone in three human cell lines, indicating that it is not an event specific to a certain inducer or a certain cell type. By contrast, UV at 300 or 320 nm showed only a marginal effect. UV at 280 nm was likely to block MT gene transcription because Cd-induced increase of MT mRNA was strongly inhibited by irradiation. Cd induction of 70-kDa heat shock protein mRNA was also inhibited by UV irradiation, suggesting that the expression of inducible genes are commonly sensitive to UV. Furthermore, we observed that the irradiation of UV at 280 nm renders NB1RGB cells extremely susceptible to Cd, probably due to the reduced MT synthesis. These observations strongly suggest that UV at 280 nm severely damages cellular inducible protective functions, warning us of a new risk of UV exposure.

Yamada, T., et al. (1995). "A mutant strain (LEC) of rat with low degree of zinc-induced hepatic metallothionein gene expression." Life Sci 57(16): 1515-1524.

A mutant strain (LEC) of rats was found to possess the feature of low degree of the zinc-induced hepatic metallothionein (MT) gene expression due to an alteration of the transcription factor concerned in the gene expression. Northern blot analyses showed that the amount of MT-1 mRNA induced by intraperitoneal zinc injection is smaller in LEC mutant rat liver than in normal rat liver, while the amount of MT-1 mRNA induced by copper injection is indistinguishable between LEC and normal rat livers. Gel retardation assays showed that LEC and normal rat livers are different in the nuclear protein which binds to the metal-responsive element (MRE) of the MT gene in a zinc-dependent manner, and that the efficiency of the zinc-dependent binding of the nuclear protein to the MRE is lower in LEC rat liver than in normal rat liver. LEC rat should provide a useful model to understand the transcription factor concerned in the MT gene expression by zinc.

Yamada, T., et al. (1992). "Elevation of metallothionein gene expression associated with hepatic copper accumulation in Long-Evans Cinnamon mutant rat." Biochim Biophys Acta 1131(2): 188-191.

The mechanism of the metallothionein (MT) gene expression was investigated in a mutant rat, LEC, which exhibits an abnormal accumulation of copper in hepatocytes. The levels of MT mRNA were extremely high and correlated with the hepatic copper concentrations in LEC rat liver. Gel retardation assays in nuclear extracts from LEC rat liver showed an increase in the copper-dependent binding proteins, which bind to the metal responsive element (MRE) of the MT gene. These results suggest that the high intracellular copper accumulation results in the elevation of the MT gene expression through

increasing a putative trans-activating factor in LEC rat.
Yamada-Okabe, T., et al. (1995). "Effects of oncogenes on the resistance to cis-diamminedichloroplatinum(II) and metallothionein gene expression." Toxicol Appl Pharmacol 133(2): 233-238.

Transformation of NIH3T3 cells with the ras, the sis, or the neu oncogene rendered cells less susceptible to cis-diamminedichloroplatinum(II). Since resistance to cis-diamminedichloroplatinum(II) is reported to be associated with increased levels of metallothionein, we examined effects of these oncogenes on metallothionein gene expression. NIH3T3 cells were first transfected with the lacZ gene whose transcription is under the control of mouse metallothionein I promoter and then with the ras, the sis, or the neu oncogene. The ras and the sis oncogenes increased beta-galactosidase activities which were induced either by metal (cadmium and zinc) or by glucocorticoid (dexamethasone), whereas the neu oncogene repressed its activity. When SV40 early promoter was used instead of metallothionein I promoter for the lacZ gene transcription, the beta-galactosidase activities were not affected by metal, dexamethasone, or any of these oncogenes. This result was coincident with that of reverse transcription polymerase chain reaction that metal-induced MT I mRNA was only detected in the sis- or the ras-transformed cells, whereas any of these oncogenes did not affect the metal-induced transcription of the MT II gene. These results demonstrate that the ras and the sis oncogenes upregulate the metal- or glucocorticoid-induced transcription from metallothionein I promoter, but the neu oncogene negatively regulates it. Thus, resistance to the chemotherapeutic agent by oncogenic transformation is partly associated with the metallothionein gene expression, and MT I and MT II gene expressions are differently controlled by different oncogenes.
Zafarullah, M., et al. (1989). "Endogenous and heavy-metal-ion-induced metallothionein gene expression in salmonid tissues and cell lines." Gene 83(1): 85-93.

Endogenous levels of metallothionein (MT) mRNA were detected by RNA probes in several somatic and germ-line tissues of rainbow trout, such as eggs, ovaries and immature testis. These levels may be related to metal-ion homeostasis in the observed tissues. The induction kinetics of trout MT isoform B (MT-B) mRNA were studied after single intraperitoneal injections of CdCl2, CuCl2 and ZnCl2. MT-B mRNA was induced within 12 h in liver, kidney, spleen and gills. However, over the 48-h experimental period, the kinetics of MT-B mRNA accumulation differed in response to the three metal salts, possibly due to differential handling of the salts by these tissues. Multiple metal-salt injections induced high levels of MT-B mRNA in the four tissues studied. In the rainbow trout hepatoma cell line, ZnCl2 was a better inducer of the MT-B gene, as compared to CdCl2 and CuCl2. The expression of the exogenous trout MT-B promoter in Chinook salmon embryonic cell line indicates the presence of MT regulatory factors. In contrast, the endogenous MT genes in these cells are quiescent, possibly due to the methylation of their promoter region.

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