

Forgotten partners and function regulators of inducible metallothioneins

Pavić, Mirela; Turčić, Petra; Ljubojević, Marija

Source / Izvornik: *Arhiv za higijenu rada i toksikologiju*, 2019, 70, 256 - 263

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

<https://doi.org/10.2478/aiht-2019-70-3317>

Permanent link / Trajna poveznica: <https://urn.nsk.hr/urn:nbn:hr:163:910358>

Rights / Prava: [In copyright](#)

Download date / Datum preuzimanja: **2022-06-28**



Repository / Repozitorij:

[Repository of Faculty of Pharmacy and Biochemistry University of Zagreb](#)



Forgotten partners and function regulators of inducible metallothioneins

Mirela Pavić¹, Petra Turčić², and Marija Ljubojević³

¹ Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

² Department of Pharmacology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

³ Molecular Toxicology Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia

[Received in July 2019; Similarity Check in July 2019; Accepted in November 2019]

Metallothioneins are peculiar cysteine rich, heat resistant, small cellular plasma proteins expressed through almost all life forms. The currently established biological functions of metallothioneins are the homeostasis of essential metals and protection against toxic transitional metals (TM) alongside defence from oxidative stress by direct scavenging of reactive oxygen and nitrogen species (ROS and RNS). In mammals, among the four main evolutionary conserved forms, only the ubiquitously expressed metallothionein 1 and 2 (here abbreviated as MT) are inducible by TM, oxidative stress, glucocorticoids and starvation among various other stimuli. However, more than sixty years after being discovered, metallothioneins still bear unresolved issues about their possible physiological function and regulation. The biological function of MTs has still not been associated with the *in vitro*-demonstrated capacity of MT interaction with cellular molecules glutathione (GSH) or adenosine triphosphate (ATP), or with the possibility of direct iron-MT binding in the reducing intracellular environment of some organelles, e.g. lysosomes. Iron as the most abundant cellular TM is also one of the main physiological sources of ROS. Moreover, iron exhibits strain, sex and age differences that reflected ROS generation and MT induction in (patho)physiology and toxicology studies. A recent study showed that iron sex differences follows expression of both ferritin and MT leading to wide implications from essential TM interconnectivity to aging. This review places emphasis on biochemically proven but physiologically ignored interactions of MT with iron to stimulate advanced research for establishing a wide frame of the biological roles of MTs important for health and longevity.

KEY WORDS: adenosine triphosphate; aging; copper; ferritin; glutathione; iron; oxidative stress; sex differences; steroid hormones; transition metals; zinc

GENERAL INTRODUCTION TO VERTEBRATE METALLOTHIONEINS

In the six decades of research since metallothioneins were first isolated (1), over 12,500 biomedical publications have been indexed in PubMed (October 2019) about these atypical metal binding proteins, and almost half as many more if other scientific databases are considered (2).

Structurally, mammalian metallothioneins are heat resistant, small (about 6–7 kDa), dumbbell shaped proteins with the ability of its N-terminal beta domain to bind labile and easily exchangeable three divalent (or more monovalent) TMs through 9 cysteines (Cys) and of its C-terminal alpha domain to bind more stable four divalent TMs coordinated with 11 Cys, whereas cleft-formed through a linker region between two domains can potentially accommodate phosphate, GSH or ATP molecules (Figure 1) (2-8). From around 60 amino acids (AA) in mammalian metallothioneins, one third are Cys AA and a significant part are made of conserved lysine and serine without the presence of any aromatic or histidine AA. Mammals have four main forms

or family members of metallothioneins among which MT1 and MT2 (MT), of primary concern in this review, are ubiquitously present because of their cellular protective task and the fact that their expression is induced by various stresses, e.g. heat shock, glucocorticoids, oxidative stress, calorie restriction (CR) as well as with a wide range of TMs. Metallothionein 3 (MT3) has a specific function in the central nervous system (CNS) as a growth inhibitor factor (GIF), while metallothionein 4 (MT4) is found only in squamous epithelial cells (2-4).

Functionally, MTs are involved in the protection against oxidative stress as well as in the homeostasis of essential TMs with stress placed on zinc (Zn) and copper (Cu). Additional metal sequestration and defence against toxic TMs, e.g. cadmium (Cd), mercury (Hg), silver (Ag), platinum (Pt) and others such as lead (Pb) and metalloids such as arsenic (As), are accompanied by protection against oxidative stress through direct ROS and RNS binding (9-12). The biochemical work done thus far has revealed non-cooperative binding of various metals to MT according to their thiol affinity including iron (Fe) (10). This review emphasizes the possible biological role of iron-MT binding from *in vitro* findings through the 1980s, which were largely ignored despite the detection of iron in the first MT isolate by Vallee (1). Throughout evolution, we are bounded to the

Corresponding author: Marija Ljubojević, PhD, Molecular Toxicology Unit, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10000 Zagreb, Croatia, E-mail: mljubo@imi.hr

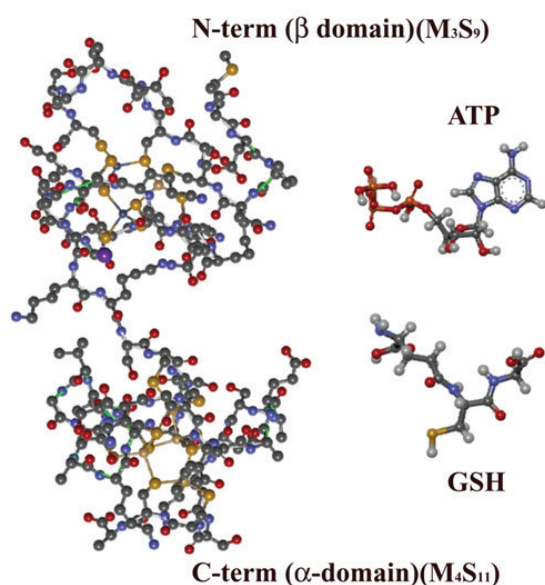


Figure 1 Molecular structures of MT (4mt2, PDB), ATP, and GSH. Using the Discovery Studio 2019 Client (BioVia/Accelrys, San Diego, USA), the superposition of real conformations of whole MT and molecules ATP and GSH was performed. This showed a considerable possibility of binding into the interdomain cleft of MT with a probability of pH dependence that influenced the intradomain linker region (68). N and C terminal domains are shown with their metal binding properties: M – transition metal (divalent), S – thiol moiety of cysteine (gold yellow colour)

trio of transition metals iron, zinc and copper that seems to reflect in metallothionein's properties and their presence in today's organisms (2, 4)

No MT is indispensable for life, so after knocking out MT1 and/or MT2, experimental animals (MT null) are viable but exceedingly sensitive to various stresses and prone to developing certain unexpected consequences of metabolic impairment and shorter lifespan, together with ionizing and UV radiation sensitivity. Conversely, animals that overexpress MT do have higher resistance to various stresses and live a longer and healthier life (13-15).

The aim of this review was to motivate researchers in the field for more integrated and/or system biology-oriented work on the involvement of MTs in the fine-tuning of essential TMs as it is well-known that zinc is necessary for antioxidative and anti-inflammatory action together with Fenton reactive copper and iron that jointly affect metabolism regulation throughout aging (16, 17).

OXIDATIVE STRESS AND TMs

The relevant literature comprises volumes of studies on antioxidative actions (16-19). However, it is not so often emphasized that iron, the most abundant TM in the organism, is a strong and potent generator of physiological oxidative stress, through valence change according to the environment from reduced divalent ferrous ion (Fe^{2+}) to oxidized ferric ions (Fe^{3+}). By releasing an electron in the presence of hydrogen peroxide (H_2O_2), ferrous ion initiates

a Fenton reaction of hydroxide radical (OH^\cdot) generation that modifies all biomolecules. Despite being mainly considered through oxygen delivery via haemoglobin in erythrocytes and myoglobin in muscles, iron should also be viewed through the lens of its abundance in the cells of various organs and importance in all biological processes as in the mitochondrial electron transport chain together with its role in enzyme functions, e.g. catalase (CAT), and structure, e.g. S-Fe clusters in ribonucleotide reductase (RNR). Specificity in iron turnover throughout the organism, organs, and cells resides in the regulated mechanisms of Fe accumulation without any processes of active excretions. Cellular protection from the negative impact of Fe relies on a very small group of potential cytosolic iron chaperones together with the great storage capacity of the ferritin nanocage and may include the possibility of Fe-MT interaction and iron scavenging in organelles. The mentioned roles in various metabolic functions make iron an essential TM in all organisms, while both its deficiency and overload may lead to serious health problems (17).

When describing the function and structure of various metalloproteins, with a few exceptions, focus is often placed on Zn as the second most abundant TM in the organism. Zinc is of immense importance for about 3,000 proteins because of its redox inertia and importance for the structure and function of enzymes and transcription factors. Zn is known to protect from various kinds of stress, as demonstrated by numerous studies in humans and animal models. Furthermore, the upregulation of MTs fulfils Zn's antioxidant function and together with anti-inflammatory and other positive and protective roles, it is undoubtedly important for health and longevity (16, 19).

The third essential TM present in organisms in abundance is Cu, which as a Fenton reactive metal must also be tightly controlled mainly by MTs and a few chaperones in the cell. An important role in the regulation of copper levels is also attributed to the copper plasma carrier ceruloplasmin, a multicopper ferroxidase that together with another copper enzyme hephaestin is involved in the iron transport metabolism (17). Other TMs that belong to trace or ultra-trace elements in the organism are present at concentrations a few orders of magnitude lower and have a place in the metalloprotein networks that include MTs (20).

MEMBRANE TRANSPORT OF IRON, ZINC, AND COPPER

Membranes of cells and organelles are barriers for TMs. Currently, the transport of all essential TMs is covered either through the general divalent metal transporter (DMT1; Solute carrier transporter, SLC11A2) or through active gastrointestinal ATP7A and hepatic ATP7B transporters for copper and ZnT (SLC30A) and ZIP (SLC39A) transporters

for zinc and iron to the only cellular iron export protein ferroportin FPN1 (SLC40A1) (20).

Many of the reviews published thus far have described in detail a vast amount of knowledge, thereby creating a complex picture of the transport mechanism for each TM to the point of its autoregulation through feedback loops that are still to be discovered (21-26). After years of research on zinc transporters, two large families of divalent metals emerged; nine ZnT family members that facilitate entry into the cytosol and fourteen ZIP family members that enable exit. This excessive number of zinc transporters may be the reason why MT null animals can be viable, as some of the transporters are essential for embryonal development onward. Both types of transporters carry not only Zn, but also cadmium (Cd) and manganese (Mn), while ZIP members can transport Fe as well. Some members of the ZIP family, named after the zinc and iron regulated transport proteins found in plants (Zrt/Irt-like proteins), do have a relevant physiological role in non-transferrin bound iron (NTBI) transport and current research into these is very active.

CELLULAR DISTRIBUTION OF MTs

MTs as TM chaperones and ROS/RNS quenchers play an important role in cellular metabolic tangled webs. They, however, are not located exclusively in the cytosol (2-4, 9-12); MT presence has often been observed in the mitochondria and nucleus of proliferative cells and within lysosomes. Furthermore, each cellular compartment has its specificity regarding function and microenvironmental conditions, especially proton abundance that defines pH (27).

The cytosol can be regarded as a pH neutral main milieu for all organelles, proteins, and other components that interact with TMs. This enables metals to be stored, utilized or redistributed, in parallel with the dynamic level of ATP and GSH concentrations that regulate signalling of a cell's energy and oxidative status, respectively.

Mitochondria, as the main energy supply compartment, are also constant producers of ROS in the form of superoxide radicals (O_2^-) that are reduced to H_2O_2 through superoxide dismutase (SOD) activity. This organelle is very rich in Fe enzymes under oxidative conditions, and has a specific compartmentalization and inner structure with two levels of proton concentrations. The inside of the mitochondrial matrix is an alkaline environment suitable for citric acid cycle and hem synthesis, whereas the intermembrane space has an only slightly lower pH than the cytosol. Mitochondria are also involved in the process of programmed cell death (PCD) by mediating classical intrinsic pathways of apoptosis with cytochrome c release and caspase activation. However, under certain conditions of metabolic disturbance that liberates too much iron in the presence of H_2O_2 consequently generating lipid peroxides,

mitochondria can also be involved in another pathway of non-apoptotic regulated cell death (RCD) called ferroptosis (28). MT has a proven protective role that can be primarily linked to the prevention of either the causes or consequences of ROS/RNS action (2-4), but whether MT's anti-apoptotic and anti-ferroptotic role can also involve the direct binding of free Fe, e.g. from lysosomes or damaged mitochondria is still an open question.

The nucleus is a cellular compartment where MTs can mostly be found during the cellular proliferative phase, when MTs serve probably as donors of TMs, especially of Zn to transcription factors (steroid hormone receptors and zinc finger transcription factors), but also possibly as direct ROS/RNS scavengers.

Lysosomes are main organelles where degradation of cellular MT protein occurs through acid proteases cathepsins when TM-MT complexes are much more resistant to degradation than MT without a bound TM (apothionein). That degradation scenario is unusual for free cellular cytoplasmic proteins, as it largely does go through the proteasomal system (29).

A cellular recycling process through lysosomal degradation of organelles and proteins rich in Fe make one unexpected interaction milieu of MTs and Fe (30, 31), which attracted the attention of researchers in the last decades. The findings by Baird et al. (31) suggest that the acidic environment of lysosomes is a surrounding that can stimulate MT release of Zn or Cu while facilitating Fe-MT binding and thereby protecting organelles from ROS generation from liberated reduced Fe in the presence of H_2O_2 . Fe endosomal/lysosomal entrance also occurs with transferrin-Fe (TfFe) cellular arrival throughout receptor-mediated endocytosis (RME), autophagocytosed mitochondria, and other organelles as well as through a special autophagic process called ferritinophagy of Fe stored in ferritin, all of which use endosomes through lysosome compartments for releasing and mobilizing Fe, and consequently creating a cytosolic liable iron pool (LIP) (17, 30, 31). In this process MTs from cytosol enter the lysosome by microautophagy where they acquire higher affinity for reduced Fe and participate in its sequestration, preventing uncontrolled Fenton reactions and Fe release through a Zip8 or DMT1 into the cytosol and averting the overall LIP increase.

In relation to interference with normal physiology, the example of the well-studied Cd toxicity shows that exogenous toxic TM can induce MTs and generate oxidative stress. As Cd is not a redox reactive (Fenton active) TM, it can enter the cell through molecular mimicry to interfere with described endocytic pathways and liberate Fe which triggers oxidative damage (10).

Recently, the role of lysosomes in the aging theories also highlighted the importance of iron in autophagocytic pathways through an increase in LIP or undegraded protein accumulation and overall ROS generation (32, 33).

DIRECT INTERACTION OF IRON AND MTs

The established function of Zn and Cu homeostasis in the organism is based on the capacity of inducible MTs to bind various TMs with or without a possible biological function, on the basis of their –SH affinity, ion radius, orbital conformation and probably depending on environmental pH (2-4, 9-12). With this in mind, the potential of Fe-MT binding that may occur in acidic environments in lysosomes and inner mitochondrial membrane space is largely underestimated. Despite the fact that Fe was found in the first MT isolates, Fe went “under the radar” in MT research soon afterwards (1, 10, 12). A possible cause for this may be a coincidental technical finding from our group that most Fe-MT interactions involve cellular organelles and that fractions are mainly lost during protein purification through high-speed centrifugations.

From a historical perspective, the coincidence that Yutaka Kojima, who was an appreciated and a long term collaborator of Vallee and Kagi, the “fathers” of MTs, shares his surname with Nakao Kojima, the author of the first negative *in vitro* Fe-MT binding results (34), might have had at least some measure of influence on other researchers taking Nakao Kojima’s findings “for granted” and not testing the validity of these results further. Only three years later, Good and Vasak (35) published positive *in vitro* Fe-MT binding results starting from an acid to alkali environment with a subsequent multifaceted analysis of the formation, stability, and structure of the Fe-MT complex (36, 37). However, the biological relevance of these results was completely neglected in the MT-related papers that followed (3, 9), even in spite of indicative *in vivo* results observed in an avian model (39). One reason why MTs were sidestepped may be that another ubiquitously abundant and physiologically relevant Fe-binding and storage complex - ferritin – had already been isolated from the cytosol (30) and received more attention.

In vitro studies of Fe-MT binding sparsely continued throughout the 1990s among which Kennedy et al. (39) revealed electron spin resonance (ESR) spectroscopy findings that nitric oxide (NO) removes Zn from MT and interacts with freshly bound Fe-MT, while Ding et al. (40) repeated previous studies of Fe-MT binding in yeast MT. The only recent study in the 21st century explored the reduction potential of the tetranuclear iron core in the synthetic alpha domain of MTs in an alkaline pH environment. This was biochemically interesting, but physiologically not as relevant mainly because of the high pH in the experiments (41).

Another two studies on unexpected iron MT interaction (42, 43) investigated cytosolic ferritin direct contact with MTs where both Zn and Fe were released and this might well be extremely significant for MT, Zn, and Fe physiology.

REGULATION OF MT EXPRESSION

The TM driven pathway of MT expression regulation is already textbook knowledge; it goes through direct induction when another TM has higher affinity for MT binding and easily releases Zn into the cytosol. Released Zn binds to the metal transcription factor (MTF1) that, once it is activated, starts transcription through the presence of a metal response element (MRE) in the MT promoter region. Nascent MT mRNA expression is followed by translation to proteins that may increase several-fold in the abundance of mRNA templates. Oxidative stress and the glucocorticoid-driven pathway can also directly induce MT expression through respective antioxidative and glucocorticoid response elements (ARE and GRE) in the promoter region of MT genes (2-4, 9-12, 44). Noteworthy of mentioning is the fact that ferritin can be, among other protective metalloproteins, also induced with oxidative stress through common transcription factor NRF2 (nuclear factor erythroid 2-related factor 2), which acts through the ARE region in the promoter making these two metal binding proteins among the first line of antioxidant cellular defence (30, 44). Of particular note is the information that specific RCD ferroptosis induction through sorafenib interference with cysteine transport for GSH synthesis emerged as a side effect in recent studies involving this antineoplastic multiple kinase inhibitor’s mode of action in hepatocellular carcinoma (HCC). HCC cells can escape ferroptotic death and become resistant to sorafenib by inducing specific isoform MT-1G and possible other members of the MT family through the action of NRF2/ARE binding resulting in consequent ROS scavenging, which for now seems to be without changes in free iron abundance (28, 44, 45).

Furthermore, from the gene regulation point of view, *in vitro* studies have unexpectedly shown that the GRE present in an MT promoter region can be used by progesterone sex steroid hormone receptors (46). That may be one of the pathways which affect *in vivo* physiological sex differences in the abundance of MT expression that follows sex differences in TM, especially in Fe (47, 48). The question of sex steroid hormone MT regulation is still unresolved. Much confusion has stemmed from two decades of Cd toxicity studies where protection was achieved through MT upregulation following any steroid treatment in rodents. Steroid hormone regulation influences the response to various inducers, not only to toxic TMs that have shown different effects between sexes and, moreover, between strains (49). Again, studies on sex differences that analysed Fe, ferritin, and MT and had similar findings suggest that there is a possibility that a different genetic background, which determines iron accumulation pathways including sex differences, is present in different mammals and even animal strains. This was supported by findings of Hahn et al. (50) in mouse liver and is most likely responsible for the above mentioned discrepancies reported in the relevant literature.

Although physiologically positive effects of missing male sex hormones and negative effects of missing female sex hormones in MT expression and iron liver accumulation have been observed, immediate causes for these sex differences have not been established (48). One explanation could be that MT expression respond to oxidative stress and simply follows the Fe accumulation (48, 51) that is regulated by the liver hormone hepcidin under steroid hormone control (52, 53). Consequently, MT sex differences can be directly linked to Fe, through Fe-MT binding during lysosomal degradation and TM exchange that releases Zn for MTF-1 activation and MT expression, or indirectly, through LIP that causes oxidative stress pursued by the induction of MT expression but also of ferritin for iron storage, which makes these two proteins interdependent.

GENETICALLY MODIFIED ANIMALS AND AGING

As described in a detailed review by Mocchegiani (14), the difference between MT transgenic and null animals indicates that MT fine tuning is necessary for longevity (15, 54-56). Among other considerations, the review stated that the observed anti-aging effect of upregulated MTs can be connected to factors so obvious as oxidative stress regulation and higher Zn accumulation/storage capacity that is necessary for all aspect of health, considering Zn antioxidant and anti-inflammatory function, but with the issue of the bioavailability of zinc when MT is high (14, 16, 19). Findings in MT null animals suggest that MTs are generally not necessary for the overall Zn metabolism that is possibly mediated by many redundant transport mechanisms. However, Zn is necessary for the MT metabolism, and their delicate mutual relationship is certainly among those reflected in the benefits that transgenic animals have and problems during the lifetime of MT null animals (54-56).

None of the existing literature sources mentions the possibility that an abundance of MT in transgenic animals may serve as protection from free Fe.

FURTHER CONSIDERATIONS

Essential TMs are inevitably interconnected and MTs may be the common binding proteins for their fine tuning. However, what is currently absent is a parallel comparison of changes of all three essential TMs together with their specific and common proteins for transport, regulation, carriers, and storage to firmly establish every possible interrelation (22).

The concept that GSH abundance is probably the main chaperon of iron in LIP, and a direct association between GSH and iron metabolism most visible in the process of RCD ferroptosis opens a wider perspective from both the physiological and toxicological points of view (28, 45, 57).

Biochemical *in vitro* studies (6, 58) have mainly considered Zn-regulated release from MTs depending on reduced vs oxidised GSH in that process.

Recent data pointed out sex differences even in constitutive autophagy (59) that includes ferritinophagy, as a special autophagy form of ferritin degradation, through nuclear receptor coactivator 4 (NCOA4, known as androgen receptor-specific coactivator ARA70) that directly or through protein interaction may be dependent on steroid hormones (60). Also, ferritinophagy may as well as autophagy, if not regulated and coordinated with protective mechanisms that involve GPX4 activity through use of GSH, lead to iron-mediated ROS induction that oxidizes lipids and causes ferroptosis as a form of non-apoptotic RCD (28, 45).

Moreover, Fe is not the only TM that has the ability of being stored in a ferritin multisubunit nanocage; others do as well, including essential and toxic metals (61), which is why the two metalloproteins, ferritin and MT, should be considered together. Additional indirect links of Fe and Zn metabolism that may be finely tuned by MTs are confirmed by transcription factor MTF1, indispensable to an organism, which regulates the transcription of the cellular Fe-export protein FPN1 and the plasma Fe transport protein transferrin (Tf), as well as other TM-regulating proteins, involved in Fe, Zn, and Cu homeostasis (62).

At the cellular biochemical fine tuning level, it seems that apart from the presence of ferritin storage, easily upregulated MTs may be one of the crucial small ubiquitously abundant proteins that protect cells against highly reactive Fe ions either just as oxidative quenchers or with an additional role by directly scavenging iron in lysosomes and mitochondria (31).

Moreover, MT1 does make a response to iron deficiency in the blood and tissue connecting MTs not only to storage/scavenge but also to erythropoiesis that has strong opposition/competition from the involvement of other proteins (63, 64). Nonetheless, although the above mentioned sex difference in Fe, connected to ferritin expression (65) have been known for decades, their consequences also have long been ignored and have undoubtedly influenced physiological and very probably toxicological studies (49) together with analyses of MT expression.

This review indicates that Fe (30-33, 35-40), ATP, and GSH (5-8, 58) act together with MTs alongside established protein interactions with emphasis on ferritin (42, 43, 58, 66) and that this takes place with the influence of steroid hormones in the background (Figure 2). Even as a study by Zanger and Armitage (67) which failed to prove MT and ATP interactions nevertheless revealed changes in pH when ATP is added to the reaction. These changes yielded a result regarding oxidative and pH dependence of interactions that was confirmed in a later study (8). Furthermore, there is a probability that *in vivo* accumulation of phosphate by aging may disturb ATP/GSH-MT binding position and promote

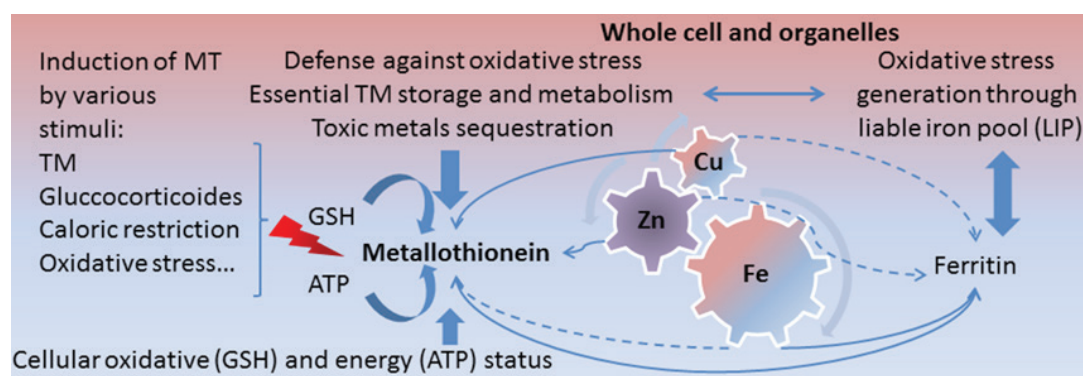


Figure 2 Concept of MT interactions with three essential TMs with sex differences indicated by red/blue gradient and with Zn as the exception in purple. The scheme also implicates that both ferritin and MT bind all of the three essential TMs in real conditions, as well as a pronounced possibility of MT-GSH and ATP interaction

multimerization/crystallization of MTs that may be involved in lipofuscin/protein aggregate formation and the pathology of neurodegenerative diseases (32, 33, 58, 68, 69).

CONCLUDING REMARKS

This review was motivated by the necessity for an *in vitro* and *in vivo* revalidation of the biologically important regulation and interactions of MT with iron. Therefore, we underline that each separate cell organelle with a distinctive microenvironment and discrete ATP and GSH content contains MTs with possible different TMs bound to them for exchange within other compartments. Emphasis should be placed not only on MT's ROS/RNS scavenging function, but also on the possibility of direct MT interaction with metals, including iron, ATP, and GSH in different cellular compartments especially mitochondria and lysosomes. TM abundance and distribution depends on physiological conditions such as sex differences and possibly even the female sex cycle regulated by steroid hormones. Sex differences in Fenton reactive TM are under regulatory mechanisms that also drive MT expression. Zinc as a sex-independent and Fenton inert TM is a highly abundant and indispensable regulatory and stimulatory factor for various proteins including MT that leads to lower oxidative stress and anti-inflammation processes. Furthermore, the possibility that MT expression can be modulated by non-toxic Zn supplementation and influence an organism's response to oxidative stress, as in Wilson's disease (70), can be applied to more disorders where oxidative stress and metal accumulation underlie the causes and/or the consequences of pathology.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgements

The author ML thanks Goran Šinko, PhD from Biochemistry and Organic Analytical Chemistry Unit for

assistance in using the computational program Discovery Studio. This work was supported by the Ministry of Science and Education of the Republic of Croatia Program and by grant IP-11-2013-1481 (AGEMETAR) from the Croatian Science Foundation for ML.

REFERENCES

- Vallee BL. Metallothionein: historical review and perspectives. In: Kägi JHR, Nordberg M, editors. Metallothionein. Experientia Supplementum. Vol. 34. Basel: Birkhäuser; 1979. p. 19-39. doi: 10.1007/978-3-0348-6493-0_1
- Capdevila M, Bofill R, Palacios Ò, Atrian S. State-of-the-art of metallothioneins at the beginning of the 21st century. *Coord Chem Rev* 2012;256:46-62. doi: 10.1016/j.ccr.2011.07.006
- Hidalgo J, Chung R, Penkowa M, Vasak M. Structure and function of vertebrate metallothioneins. In: Sigel A, Sigel H, Sigel RKO, editors. Metallothioneins and related chelators: Metal ions in life sciences. Cambridge: RSC Publishing; 2009. p. 279-317. doi: 10.1039/9781847558992-00279
- Ziller A, Fraissinet-Tachet L. Metallothionein diversity and distribution in the tree of life: a multifunctional protein. *Metallomics* 2018;10:1549-59. doi: 10.1039/c8mt00165k
- Jiang L-J, Maret W, Vallee BL. The glutathione redox couple modulates zinc transfer from metallothionein to zinc-depleted sorbitol dehydrogenase. *Proc Natl Acad Sci U S A* 1998;95:3483-8. doi: 10.1073/pnas.95.7.3483
- Chen Y, Maret W. Catalytic selenols couple the redox cycles of metallothionein and glutathione. *Eur J Biochem* 2001;268:3346-53. doi: 10.1046/j.1432-1327.2001.02250.x
- Jiang L-J, Maret W, Vallee BL. The ATP-metallothionein complex. *Proc Natl Acad Sci U S A* 1998;95:9146-9. doi: 10.1073/pnas.95.16.9146
- Maret W, Heffron G, Hill HA, Djuricic D, Jiang L-J, Vallee BL. The ATP/metallothionein interaction: NMR and STM. *Biochemistry* 2002;41:1689-94. doi: 10.1021/bi0116083
- Romero-Isart N, Vasák M. Advances in the structure and chemistry of metallothioneins. *J Inorg Biochem* 2002;88:388-96. doi: 10.1016/S0162-0134(01)00347-6
- Sabolić I, Breljak D, Škarica M, Herak-Kramberger CM. Role of metallothionein in cadmium traffic and toxicity in kidneys and other mammalian organs. *BioMetals* 2010;23:897-926. doi: 10.1007/s10534-010-9351-z

11. Wong DL, Merrifield-MacRae ME, Stillman MJ. Lead(II) binding in metallothioneins. In: Sigel A, Sigel H, Sigel R, editors. Lead - Its effects on environment and health. Berlin, Boston: De Gruyter; 2017. p. 241-70. doi: 10.1515/9783110434330-009
12. Bell SG, Vallee BL. The metallothionein/thionein system: an oxidoreductive metabolic zinc link. *Chembiochem* 2009;10:55-62. doi: 10.1002/cbic.200800511
13. Cai L, Satoh M, Tohyama C, Cherian MG. Metallothionein in radiation exposure: its induction and protective role. *Toxicology* 1999;132:85-98. doi: 10.1016/s0300-483x(98)00150-4
14. Mocchegiani E, Costarelli L, Basso A, Giacconi R, Piacenza F, Malavolta M. Metallothioneins, ageing and cellular senescence: a future therapeutic target. *Curr Pharm Des* 2013;19:1753-64. doi: 10.2174/1381612811319090022
15. Malavolta M, Orlando F, Piacenza F, Giacconi R, Costarelli L, Basso A, Lucarini G, Pierpaoli E, Provinciali M. Metallothioneins, longevity and cancer: Comment on "Deficiency of metallothionein-1 and -2 genes shortens the lifespan of the 129/Sv mouse strain". *Exp Gerontol* 2016;73:28-30. doi: 10.1016/j.exger.2015.11.014
16. Jarosz M, Olbert M, Wyszogrodzka G, Młyniec K, Librowski T. Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF- κ B signaling. *Inflammopharmacology* 2017;25:11-24. doi: 10.1007/s10787-017-0309-4
17. Muckenthaler MU, Rivella S, Hentze MW, Galy B. A red carpet for iron metabolism. *Cell* 2017;168:344-61. <https://doi.org/10.1016/j.cell.2016.12.034>
18. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J* 2012;5:9-19. doi: 10.1097/WOX.0b013e3182439613
19. Maret W. The redox biology of redox-inert zinc ions. *Free Radic Biol Med* 2019;134:311-26. doi: 10.1016/j.freeradbiomed.2019.01.006
20. Maret W. The metals in the biological periodic system of the elements: concepts and conjectures. *Int J Mol Sci* 2016;17:pii: E66. doi: 10.3390/ijms17010066
21. Nishito Y, Kambe T. Absorption mechanisms of iron, copper, and zinc: an overview. *J Nutr Sci Vitaminol (Tokyo)* 2018;64:1-7. doi: 10.3177/jnsv.64.1
22. Zhang CC, Volkmann M, Tuma S, Stremmel W, Merle U. Metallothionein is elevated in liver and duodenum of Atp7b^(+/−) mice. *BioMetals* 2018;31:617-25. doi: 10.1007/s10534-018-0110-x
23. Kimura T, Kambe T. The functions of metallothionein and ZIP and ZnT transporters: an overview and perspective. *Int J Mol Sci* 2016;17:336. doi: 10.3390/ijms17030336
24. Hara T, Takeda TA, Takagishi T, Fukue K, Kambe T, Fukada T. Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis. *J Physiol Sci* 2017;67:283-301. doi: 10.1007/s12576-017-0521-4
25. Baltaci AK, Yuce K. Zinc transporter proteins. *Neurochem Res* 2018;43:517-30. doi: 10.1007/s11064-017-2454-y
26. Baltaci AK, Yuce K, Mogulkoc R. Zinc metabolism and metallothioneins. *Biol Trace Elem Res* 2018;183:22-31. doi: 10.1007/s12011-017-1119-7
27. Casey JR, Grinstein S, Orlowski J. Sensors and regulators of intracellular pH. *Nat Rev Mol Cell Biol* 2010;11:50-61. doi: 10.1038/nrm2820
28. Stockwell BR, Friedmann AJ, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascón S, Hatzios SK, Kagan VE, Noel K, Jiang X, Linkermann A, Murphy ME, Overholtzer M, Oyagi A, Pagnussat GC, Park J, Ran Q, Rosenfeld CS, Salmikow K, Tang D, Torti FM, Torti SV, Toyokuni S, Woerpel KA, Zhang DD. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* 2017;171:273-85. doi: 10.1016/j.cell.2017.09.021
29. Klaassen CD, Choudhuri S, McKim JM Jr, Lehman-McKeeman LD, Kershaw WC. *In vitro* and *in vivo* studies on the degradation of metallothionein. *Environ Health Perspect* 1994;102(Suppl 3):141-6. doi: 10.1289/ehp.94102s3141
30. Arosio P, Elia L, Poli M. Ferritin, cellular iron storage and regulation. *IUBMB Life* 2017;69:414-22. doi: 10.1002/iub.1621
31. Baird SK, Kurz T, Brunk UT. Metallothionein protects against oxidative stress-induced lysosomal destabilization. *Biochem J* 2006;394:275-83. doi: 10.1042/BJ20051143
32. Kurz T, Terman A, Brunk UT. Autophagy, ageing and apoptosis: the role of oxidative stress and lysosomal iron. *Arch Biochem Biophys* 2007;462:220-30. doi: 10.1016/j.abb.2007.01.013
33. Terman A, Kurz T. Lysosomal iron, iron chelation, and cell death. *Antioxid Redox Signal* 2013;18:888-98. doi: 10.1089/ars.2012.4885
34. Kojima N, Young CR, Bates GW. Failure of metallothionein to bind iron or act as an iron mobilizing agent. *Biochim Biophys Acta* 1982;716:273-5. doi: 10.1016/0304-4165(82)90278-1
35. Good M, Vasak M. Iron(II)-substituted metallothionein: evidence for the existence of iron-thiolate clusters. *Biochemistry* 1986;25:8353-6. doi: 10.1021/bi00374a003
36. Ding X, Bill E, Good M, Trautwein AX, Vašák M. Mössbauer studies on the metal-thiolate cluster formation in Fe(II)-metallothionein. *Eur J Biochem* 1988;171:711-4. doi: 10.1111/j.1432-1033.1988.tb13843.x
37. Werth MT, Johnson MK. Magnetic circular dichroism and electron paramagnetic resonance studies of iron(II)-metallothionein. *Biochemistry* 1989;28:3982-8. doi: 10.1021/bi00435a053
38. Fleet JC, Andrews GK, McCormick CC. Iron-induced metallothionein in chick liver: a rapid, route-dependent effect independent of zinc status. *J Nutr* 1990;120:1214-22. doi: 10.1093/jn/120.10.1214
39. Kennedy MC, Gan T, Antholine WE, Petering DH. Metallothionein reacts with Fe²⁺ and NO to form products with a g=2.039 ESR signal. *Biochem Biophys Res Commun* 1993;196:632-5. doi: 10.1006/bbrc.1993.2296
40. Ding XQ, Bill E, Trautwein AX, Hartmann HJ, Weser U. Mössbauer studies on iron(II)-substituted yeast metallothionein. *Eur J Biochem* 1994;223:841-5. doi: 10.1111/j.1432-1033.1994.tb19060.x
41. Sano Y, Onoda A, Sakurai R, Kitagishi H, Hayashi T. Preparation and reactivity of a tetranuclear Fe(II) core in the metallothionein α -domain. *J Inorg Biochem* 2011;105:702-8. doi: 10.1016/j.jinorgbio.2011.01.011
42. Orihuela R, Fernández B, Palacios O, Valero E, Atrian S, Watt RK, Dominguez-Vera JM, Capdevila M. Ferritin and metallothionein: dangerous liaisons. *Chem Commun* 2011;47:12155-7. doi: 10.1039/c1cc14819b
43. Carmona F, Mendoza D, Kord S, Asperti M, Arosio P, Atrian S, Capdevila M, Dominguez-Vera JM. Chemically and biologically harmless versus harmful ferritin/copper-

- metallothionein couples. *Chemistry* 2015;21:808-13. doi: 10.1002/chem.201404660
44. Raghunath A, Sundarraj K, Nagarajan R, Arfuso F, Bian J, Kumar AP, Sethi G, Perumal E. Antioxidant response elements: Discovery, classes, regulation and potential applications. *Redox Biol* 2018;17:297-314. doi: 10.1016/j.redox.2018.05.002
 45. Song Y, Yang H, Lin R, Jiang K, Wang BM. The role of ferroptosis in digestive system cancer. *Oncol Lett* 2019;18:2159-64. doi: 10.3892/ol.2019.10568
 46. Slater EP, Cato AC, Karin M, Baxter JD, Beato M. Progesterone induction of metallothionein-II_A gene expression. *Mol Endocrinol* 1988;2:485-91. doi: 10.1210/mend-2-6-485
 47. Orct T, Jurasović J, Micek V, Karaica D, Sabolić I. Macro- and microelements in the rat liver, kidneys, and brain tissues; sex differences and effect of blood removal by perfusion *in vivo*. *J Trace Elem Med Biol* 2017;40:104-11. doi: 10.1016/j.jtmb.2016.12.015
 48. Ljubojević M, Orct T, Micek V, Karaica D, Jurasović J, Brelljak D, Vrhovac Madunić I, Rašić D, Novak Jovanović I, Peraica M, Gerić M, Gajski G, Kralik Oguić S, Rogić D, Nanić L, Rubelj I, Sabolić I. Sex-dependent expression of metallothioneins MT1 and MT2 and concentrations of trace elements in rat liver and kidney tissues: Effect of gonadectomy. *J Trace Elem Med Biol* 2019;53:98-108. doi: 10.1016/j.jtmb.2019.02.010
 49. Shimada H, Hashiguchi T, Yasutake A, Waalkes MP, Imamura Y. Sexual dimorphism of cadmium-induced toxicity in rats: involvement of sex hormones. *Arch Toxicol* 2012;86:1475-80. doi: 10.1007/s00204-012-0844-0
 50. Hahn P, Song Y, Ying GS, He X, Beard J, Dunaief JL. Age-dependent and gender-specific changes in mouse tissue iron by strain. *Exp Gerontol* 2009;44:594-600. doi: 10.1016/j.exger.2009.06.006
 51. Thévenod F, Wolff NA. Iron transport in the kidney: implications for physiology and cadmium nephrotoxicity. *Metallomics* 2016;8:17-42. doi: 10.1039/c5mt00215j
 52. Kong WN, Niu QM, Ge L, Zhang N, Yan SF, Chen WB, Chang YZ, Zhao SE. Sex differences in iron status and hepcidin expression in rats. *Biol Trace Elem Res* 2014;160:258-67. doi: 10.1007/s12011-014-0051-3
 53. Sangkhae V, Nemeth E. Regulation of the iron homeostatic hormone hepcidin. *Adv Nutr* 2017;8:126-36. doi: 10.3945/an.116.013961
 54. Liu Y, Liu J, Habeebu SM, Waalkes MP, Klaassen CD. Metallothionein-I/II null mice are sensitive to chronic oral cadmium-induced nephrotoxicity. *Toxicol Sci* 2000;57:167-76. doi: 10.1093/toxsci/57.1.167
 55. Iszard RD, Liu Y, Dalton T, Andrews GK, Palmiter RD, Klaassen CD. Characterization of metallothionein-I-transgenic mice. *Toxicol Appl Pharmacol* 1995;133:305-12. doi: 10.1006/taap.1995.1155
 56. Miura N, Koizumi S. Gene expression profiles in the liver and kidney of metallothionein-null mice. *Biochem Biophys Res Commun* 2005;332:949-55. doi: 10.1016/j.bbrc.2005.05.043
 57. Kumar C, Igharia A, D'Autreaux B, Planson AG, Junot C, Godat E, Bachhawat AK, Delaunay-Moisan A, Toledano MB. Glutathione revisited: a vital function in iron metabolism and ancillary role in thiol-redox control. *EMBO J* 2011;30:2044-56. doi: 10.1038/emboj.2011.105
 58. Zalewska M, Trefon J, Milnerowicz H. The role of metallothionein interactions with other proteins. *Proteomics* 2014;14:1343-56. doi: 10.1002/pmic.201300496
 59. Oliván S, Calvo AC, Manzano R, Zaragoza P, Osta R. Sex differences in constitutive autophagy. *BioMed Res Int* 2014;2014:ID652817. doi: 10.1155/2014/652817
 60. Mancias JD, Wang X, Gygi SP, Harper JW, Kimmelman AC. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature* 2014;509:105-9. doi: 10.1038/nature13148
 61. Fleming JT, Joshi JG. Ferritin: the role of aluminium in ferritin function. *Neurobiol Aging* 1991;12:413-8. doi: 10.1016/0197-4580(91)90066-S
 62. Rutherford JC, Bird AJ. Metal-responsive transcription factors that regulate iron, zinc, and copper homeostasis in eukaryotic cells. *Eukaryot Cell* 2004;3:1-13. doi: 10.1128/EC.3.1.1-13.2004
 63. Robertson A, Morrison JN, Wood AM, Bremner I. Effects of iron deficiency on metallothionein-I concentrations in blood and tissues of rats. *J Nutr* 1989;119:439-45. doi: 10.1093/jn/119.3.439
 64. Philpott CC, Ryu MS. Special delivery: distributing iron in the cytosol of mammalian cells. *Front Pharmacol* 2014;5:173. doi: 10.3389/fphar.2014.00173
 65. Linder MC, Munro HN. Metabolic and chemical features of ferritins, a series of iron-inducible tissue proteins. *Am J Pathol* 1973;72:263-82. PMID: PMC1903991
 66. Atrian S, Capdevila M. Metallothionein-protein interactions. *Biomol Concepts* 2013;4:143-60. doi: 10.1515/bmc-2012-0049
 67. Zangger K, Öz G, Armitage IM. Re-evaluation of the binding of ATP to metallothionein. *J Biol Chem* 2000;275:7534-8. Erratum in: *J Biol Chem* 2001;276:30570. doi: 10.1074/jbc.275.11.7534
 68. Zangger K, Armitage IM. Dynamics of interdomain and intermolecular interactions in mammalian metallothioneins. *J Inorg Biochem* 2002;88:135-43. doi: 10.1016/S0162-0134(01)00379-8
 69. Kuro-o M. A potential link between phosphate and aging-lessons from Klotho-deficient mice. *Mech Ageing Dev* 2010;131:270-5. doi: 10.1016/j.mad.2010.02.008
 70. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. *Lancet* 2007;369:397-408. doi: 10.1016/S0140-6736(07)60196-2

Zaboravljeni partneri i regulatori funkcije inducibilnih metalotioneina

Metalotioneini su mali proteini stanične plazme specifični po zastupljenosti cisteina i otpornosti na toplinu, a izraženi su kroz gotovo sve oblike života. U sisavaca, među četirima glavnim evolucijski očuvanim oblicima, samo se sveprisutno izraženi metalotioneini 1 i 2 (u daljnjem tekstu MT) induciraju s različitim prijelaznim metalima (TM), oksidacijskim stresom, glukokortikoidima, gladovanjem između ostalih stimulansa. Trenutna uspostavljena uloga MT-ova u stanici je homeostaza esencijalnih i zaštita od toksičnih TM-ova, zajedno s obranom od oksidacijskoga stresa izravnim uklanjanjem reaktivnih kisikovih i dušikovih vrsta (ROS/RNS). Međutim, čak i više od šezdeset godina nakon otkrića, oko metalotioneina i dalje postoje neka neriješena pitanja o njihovoj funkciji i regulaciji. Naime, funkcija MT-ova još uvijek nije povezana s *in vitro* dokazanom sposobnošću vezanja MT-ova sa staničnim molekulama GSH-a ili ATP-a, zajedno s važnim procesom vezanja željeza MT-a u reduktivnom okolišu npr. lizosoma. Željezo kao najzastupljeniji stanični TM također je i glavni izvor ROS-a. Ujedno željezo pokazuje razlike između vrsta i sojeva te među spolovima i u starenju, koje mogu odražavati nastajanje ROS-ova i izražaj MT-ova u (pato)fiziološkim i toksikološkim istraživanjima. Promjene MT-ova koje prate razlike u željezu nedavno su dokazane i imaju široke implikacije od međusobne povezanosti esencijalnih TM-ova do starenja. Ovaj pregledni članak stavlja naglasak na biokemijski dokazane ali većinom fiziološki ignorirane interakcije *in vitro* MT-ova i željeza za poticaj naprednih istraživanja koja bi izgradila širu mrežu MT-ove biološke uloge važne za zdravlje i dugovječnost.

KLJUČNE RIJEČI: adenzinotriposfat; bakar; cink; feritin; glutation; oksidacijski stres; prijelazni metali; spolne razlike; starenje; steroidni hormoni; željezo