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Forgotten partners and function regulators of inducible metallothioneins

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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

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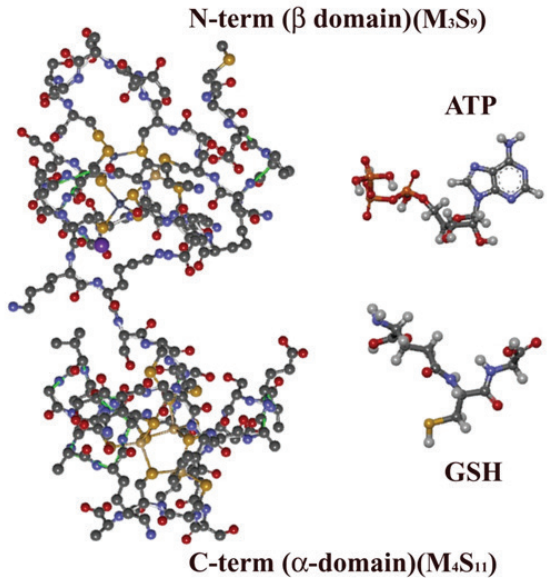

Figure 1 Molecular structures of MT (4mt2, PDB), ATP, and GSH. Using the Discovery Studio 2019 Client (BioVia/Accelrys, San Diego, USA), the superposition of real conformations of whole MT and molecules ATP and GSH was performed. This showed a considerable possibility of binding into the interdomain 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 FNP1 (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), autophagocyted 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 promotor 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 promotor 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 FNP1 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 Zangger 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

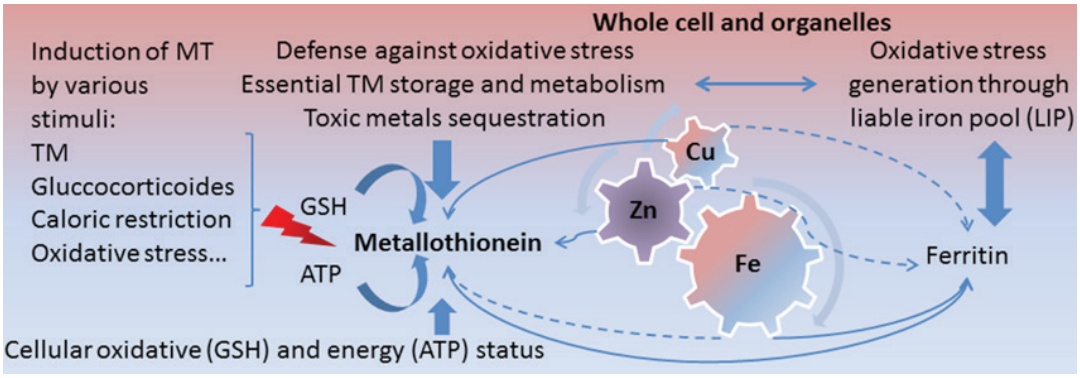

Figure 2 Concept of MT interactions with three essential TMs with sex differences indicated by red/blue gradient and with Zn as the exception in purple. The scheme also implicates that both ferritin and MT bind all of the three essential TMs in real conditions, as well as a pronounced possibility of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

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