Chapter

Metallothioneins: Diverse Protein Family to Bind Metallic Ions

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Abstract

Metallothionein's (MTs) are the lower molecular weight (6-7 kDa) proteins that are found to be present in almost all organism types ranging from prokaryotes to eukaryotes species. MT are the metal detecting proteins that can mitigate the effect caused by the excess metal ions. They are also found to be involved in cellular process such as cell growth regulation, ROS (Reactive Oxygen Species) and DNA repair. The protein was termed as Metallothionein due to the unusual higher metal (metallo) and the sulfur (thiol) content. They are further grouped into 3 classes *viz.,* class I, II and III. The Class I and II MTs are polypeptides that were obtained from direct gene products, the class III MTs are from the cysteine-rich non-translational molecules that are termed as phytochelatins. The metal ions are been sequestered through the MTs with Cys rich motifs. All the cysteines are present in the reduced form and are been co-ordinated through the mercaptide bonds. The cysteines present in the MTs are preserved across the species, it is supposed that, cysteines are essential for the function and the MTs are required for the life. Metallothionins structure, conservation in evolution, their ubiquitous nature of occurrence, the genes redundancy and the programmed MTs synthesis in development, regeneration and reproduction of living organisms are some of the weighty arguments in suspecting MTs to also serve others and perhaps the high particular metal-related cellular roles. In this chapter, there is a detailed discussion about Metallothionein its structure, occurrence and function.

Keywords: Metallothioneins, proteins, heavy metals, tolerance

1. Introduction

Many of the elements are play a significant role in all kind of organisms and pose some functional outcomes in living organisms. Based on this outcome, elements are classified as essential and non-essential elements. Recent trace element research showed many of the metals also play a major significant role in metabolisms but they required very trace quantities. The metal ions of iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), cobalt (Co) molybdenum (Mo) and the non-metals selenium (Se) and iodine (I) bromine (Br), rubidium (Rb), aluminum (Al), nickel (Ni), titanium (Ti) and barium (Ba) etc. exceeded

certain limits, it become toxic to organisms. Predicting the role of metals in living organisms is difficult since its intrinsic characteristics, redox properties, charge, and, above all, size. Toxicity of the metals mainly through altering the cell metabolism and thereby blocking synthesis of fundamental enzymes. To mitigate this, organisms have developed different detoxifying mechanisms such as blocking of toxic metals through oligopeptides or proteins and chelating metals encoding by genes, which are called metallothioneins (MTs) which possess residues of cysteine (Cys) having thiol or sulfhydryl groups (R-SH) and thereby immobilize the metallic ions. The prime function of MTs is the storage, transportation and binding of metals. Margoshes and Vallee's discovery of Cd-binding, cystein-rich protein in the horse kidney marked a birth field for the research that focused to study a novel polypeptide super family, the metallothioneins (MTs). The MTs are family consisting of low molecular weight (6-7 kDa), cysteine (Cys)-rich, non-enzymatic proteins that are found ubiquitously in animals, in higher plants, in eukaryotic microorganisms, and many prokaryotes [1, 2]. The metal ions is sequestered through MTs by binding with Cys rich metal binding – motifs Cys-Cys, Cys-X-Cys, or Cys-X-X-Cys) that [3, 4]. Based on the Cys residues arrangement, MTs grouped into different classes [5, 6]. All the cysteines present in the reduced form are been coordinated to the metal ions that gives rise to the spectroscopic appearance of the metal-thiolate groups. Cysteines in the MTs are preserved across the species, it is suspected that, cysteines are essential for the function and the MTs is required for the life. MTs have some composition of unusual amino acid as they do not contain any aromatic amino acids and the most important is that, one third of its residues contacysteines. Moreover, MTs show spectroscopic features of metal thoilates (mercaptides) [7]. MTs can bind Cd, Ni, Zn, Cu, and Pb with the affinities that depend up on the bioavailability and concentration of heavy metals. Three major scientist groups have contributed in the field of MTs include:

1. Toxicologists and physiologists for analyzing MTs role in the heavy metal metabolism and the de-toxification.

2. Protein chemists and spectroscopies intrigued by its usual structural features.

3. Molecular biologist interested in the gene regulation and use of MTs (promoter) sequences for the genetic engineering experiments.

MTs play a critical role in protecting the cell from harmful factors i.e. metals, free radicals, etc. through controlling cell growth, differentiation, proliferation, explicating their nuclear vs.cytoplasmic localization [8]. MTs is cytoplasmic protein, accumulated in lysosomes, trandpoted to the nucleus and intermediated space in mitochondria [9, 10]. MTs transported the metals from one cell to other cell by receptor- mediated mechanism and metal is transported to the cytosol but protein remains in an endocytotic compartment [11, 12]. According to Wang et al. [13], MTs have exhibits four main functions and it is illustrated in the **Figure 1**.

Nevertheless, although MTs were discovered over four decades back, their physiological functions are not fully understood still [14]. Based on the this prelude this review is being taken up to refresh the MTs structure, classification and role in metal detoxification.

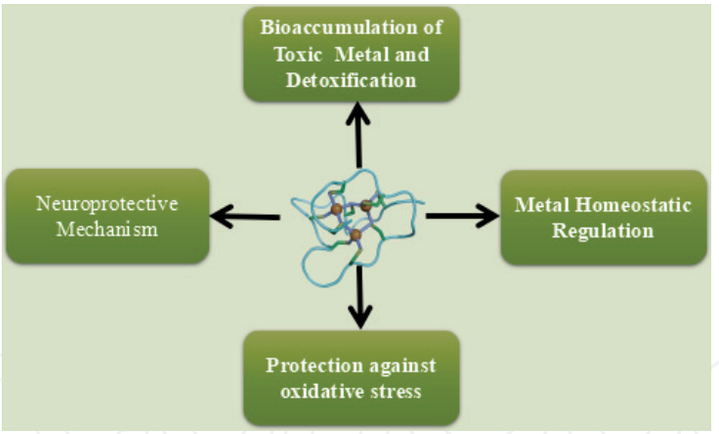

Figure 1.
MTs - four main functions. (adopted from [13]).

2. Various scientific developments in metallothionein research

The scientific research history of MTs begins in 1950s almost seven decades back. The developments in MTs research includes the contributions from scientists in fields of environmental hygiene/medicine, biochemistry, medicine, pathology, nutrition and toxicology and are best illustrated in number of articles that are been published. During this time, in protein research methods, there is a considerable development in isolation and analytical methods for identification. In beginning the interest in MTs was focused on two main topics. The major interest were in the protein chemistry and in the toxicological, *i.e*. the metabolic pathways for the metal toxicology specifically in relation to the cadmium kinetics. In the recent years, the key contributions are brought in the molecular biology. Consensus and the conclusions reached at various scientific meetings on the metallothionein are based up on the published data as depicted in the **Table 1**.

In 1957, the data related to cadmium binding protein in the equine tissue were published. However, in the beginning this study was first initiated through a report that was in a form of abstract that deals with cadmium in the human organs. In most of the animal species, cadmium is present in small amounts in tissues and the

Year	Scientific developments
1960,1961	Metallothionein
1964	Induction by cadmium
1971	Modification of Cd-toxicity
1972	Amino acid composition
1976	Sequence
1979	1st international meeting - Consesus nomenclature
1979	Radioimmunoassay
1983	International meeting - Metallothionein and nephrotoxicity
1985	2nd International meeting on metallothionein
1992	3rd International meeting on metallothionein
1996	Workshop on metallothionein

Table 1.
Various scientific events held on the metallothionein.

body fluids. For explaining this unexpected finding some of the hypotheses were postulated. Either cadmium can be present as bound to macromolecule or so had some natural function in the biological system or the cadmium also could be just a contaminant. The first report in 1960 was published on metallothionein [15]. For the first time that a cadmium containing protein that are isolated from the equine renal tissue, was termed as 'metallothionein' based on the higher content of sulfur (4.1%) followed by Cadmium (2.9%) and Zinc (0.6%). Later researchers estimated that the molecular weight was found as 10000 ± 260 Da.

The absorption at specific wavelength of 250 nm revealed the cadmium mercaptide bonds. The MTs lack an aromatic amino acid due to the non absorption at precise wavelength of 280 nm. These were further examined through amino acid analyses. Higher sulfur concentration of about 4.1% was explained through the amino acid analyses that revealed high cysteine content. The eactive protein mercapto groups at this time were examined through titration with the p-chloro mercuri benzoate (CMB), N-ethylmaleimide (NEM) and silver ions. The Amino acids were determined using both ion exchange chromatography and two-dimensional paper chromatography. The cysteine residues were found as cysteic acid after metallothionein oxidation with the performic acid and as N-ethylmaleide derivatives. The Sedimentation constant were determined by Schlieren diagram with the use of sedimentation through ultracentrifugation to 1.75 (S_{020w}). Partial specific volume, diffusion constant, and friction ratio were obtained. The estimated protein molecular weight was still varying between 9790 and 10500 Da. These were partly explained by several artifacts during the preparation. Metal analyses for Cd and Zn reported 5.9% of MTs weight and 5.2 g atoms mol^{-1} for cadmium and for zinc as 2.2% by weight and 3.3 g atoms mol^{-1}. Some exchange between Cd and Zn was taking place obviously. It was suggested that the bonding with 3 SH-groups together with an atom of either Cd and Zn was formed.

In the years between 1970s and early 1980s, there were only few research groups performed researches that are related to MTs. A workshop was arranged with nearly 25 invited participants who had submitted their background manuscripts and the tentative report [16] was prepared and were distributed in advance to each of the participant. A consensus report was agreed during the meeting held in the year of 1978.

During this Ist international meeting about MTs, held in the Zurich consensus was reached about, e.g. nomenclature and methods for protein preparing. This first meeting had been followed by another meeting held at Zurich in 1985 that were more open but still with a workshop consensus. In between, a meeting was arranged at Aberdeen in1981.

3. Structure and occurrence of metallothionein

The structure of MTs was first reported by Kagi and Schafer [15]. MTs have 2 domains consists of a cluster with three and other with 4 metal atoms. The gene are located on the chromosome 16. This protein contains a number of isoforms that are coded with several alleles. The ratio of the mRNA for MT-I and MT-II genes remains constant during the induction with metals, e.g. Cd, Cu and Zn. The MTs amino acid sequences from mammalian sources reveals that all of them contain nearly 61 amino acids that are more similar in their composition. All contain 20 cysteine residues which remain invariant to the amino acid sequence. Every cysteines do participate in the coordination of the seven moles of Zinc or cadmium per mole of the MT [17]. The MTs are the cytoplasmic proteins; but, their occurrence during fetal development in nucleus are given by immuno histochemistry. The Plant MTs were first recognized in roots of the copper-resistant strain of *Agrotis gigantean* and also in roots of the tomato

plants that are been exposed to higher concentrations of Cadmium chloride in medium are found in numerous other plants like Cd-treated maize, tobacco, wheat, rice and cabbage and also added found in some cell suspension cultures of plants [18, 19].

Metallothioneins from mammals contain 20 cysteins residues and bind seven cadmium or zinc ions in two discrete clusters. The thiolate side chains of these proteins act as bridging and terminal ligands to form Cd3SCy9 and Cd4 SCy11 clusters.

4. Classes of metallothionein

Based upon their similarities in structure, the MTs are divided into 3 classes:

Class I: This type present in mammalians, oysters, some fishes, crabs and mussels as Cys - Cys proteins.

Class II: Occurs in fungi, plants, yeasts, drosophila, cyanobacteria and nematodes in the form of Cys – x - Cys.

Class III: Presence noticed in phytochelatins that present in all plants, some fungi and also algae as Cys – x – x - Cys.

The MTs belongs to the class I & II are been produced through direct mRNA translation. Whereas, the class III MTs belongs to the metal-binding polypeptides and have peptide bonds which are biologically synthesized as some of the enzymatic products. All vertebrates that are examined contain 2 or more distinct isoforms of MT, that are grouped into 2 classes, designated as MT-I and MT-II, depending upon elution position from the DEAE cellulose. In many cases each of the class actually consists of various different proteins that were designated as MT-IA, MT-IB, MT-IC, etc.

General characteristics of Metallothioneins are,

1. MT I and MT II's molecular weight is 6-7 kDa, 61 amino acids, 20 Cysteine (30%), C-alanine, N-acetylmethionine, no histidine, no aromatics.

2. The have an unique amino acid sequence, metal clusters/tertiary structure

3. Metal content; Cu, Cd, Hg, Zn; 5-10% W/w

4. Light absorption are at 250 nm (Cd), 275 nm (Cu), 225 nm (Zn), 300 nm (Hg)

5. Induced synthesis through Zn and Cd.

6. There are no disulfide bonds, heat stability

7. Isoforms

8. Localization on chromosome and cytoplasm

MT expression in the crops serve as the key index in selecting for the heavy metal tolerance. In attempting for shedding light on their function, the researchers relied on the RNA blot hybridization for studying genes expression of MT during the development and also in response to several other environmental factors. Detailed localization of the MT mRNAs or gene promoter activity is obtained through reporter gene expression studies and *in situ* hybridization. The RT–PCR are evaluated as an alternative for Northern blot analysis for quantifying mRNA levels of MT. But the sensitivities of the two techniques were identical for detecting the induced mRNA levels in MT. Thus the above results suggests that the RT–PCR would be a quantitative and a sensitive method for evaluating gene

expression of MT [20]. Most of the MT genes are been expressed at a higher level in the plant tissues, in the terms of the transcript abundance. The direct evidence are from the rice gene profiling experiments using SAGE (serial analysis of gene expression) protocol. The transcripts from four MT genes comprised about 3% of the transcripts in 2 weeks old seedlings. Metallothionin gene belonging to Type II contributes about 1.26% of all transcripts that was found to be most expressed form in that tissue. The MT, 2 forms of transcripts as Type 3 genes counted as extra 1.25% of mRNAs. Numerous MT genes that are identified through differential screening of the cDNA libraries indicates that the RNAs encoding to MTs are higher in most of the other plant species also.

In general, affinity of the metal ions for binding sites follows the typical order of thiolate model complexes, *i.e.,* Zn (II) < Pb (II) < Cd (II) < Cu (I), Ag (I), Hg (II), Bi (III) [15]. The MT are usually detected by its virtue of high content of the metals (detected by labelling with radionuclides or by atomic absorption spectrophotometer) or by cysteine. Some of the procedures include, reversed phase High Pressure Liquid Chromatography (HPLC) gel filtration be elevated as a variety of mammalian metallothionins and utilized for immune-electrophoresis, radioimmunoassay and immunodiffusion. These protocols are mainly helpful for the detection of minimum quantity in MT. Basic structure of MT are examined by several biochemical and bio-physical techniques such as NMR spectroscopy, UV, ESR,CD partial proteolysis and X-ray crystallography.

5. Metallothionein redox cycle and function

The MT redox cycle play an important role in the MT biologic function. It might link to homeostasis of the essential metals, detoxification of the toxic metals and protection against the oxidative stress. Advancement in MT research is shown by demonstrating redox regulation of the Zn-S interaction and zinc coupling. The Zn-MT cluster structure provides some chemical basis through which the cysteine ligand induces the oxido reductive properties. These structure permits for the thermo-dynamic stability of Zn in the MT. The decrease of oxidized MT can restore Zn binding ability, thereby replicates endogenous reservoir of zinc [21]. These process constitutes the redox cycle of MT, as depicted in the **Figure 2**, that provides some new perspective upon MT biologic function.

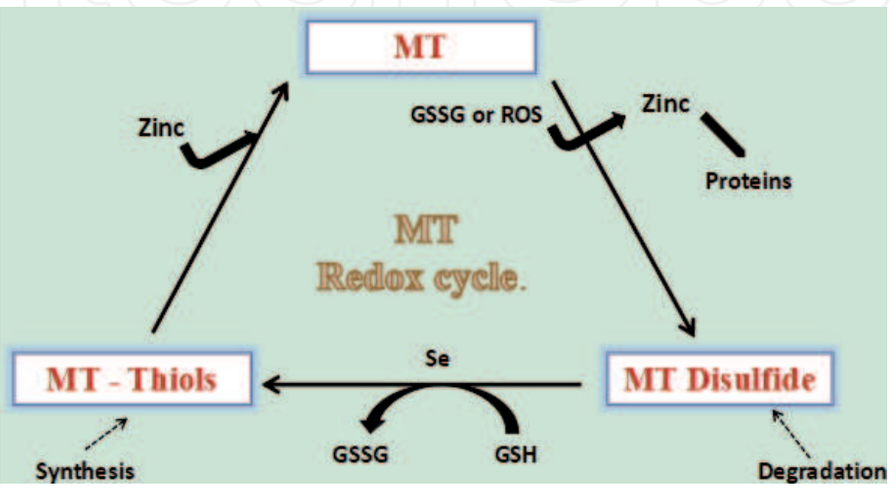

Figure 2.
Redox cycle of MT.

Under the physiologic conditions, Zn that bounds to the MT are been released from the thiolate group oxidation. The MT disulfide and/or thionin formation follows a Zn release. The MT disulfide and/or the thionin undergoes some degradation, however, when the oxidized environment gets reduced. This reduction progress is more in occurrence of a catalyst (selenium). These processes constitute the MT redox cycle, which plays a critical role in biologic function of MT.

6. Proposed mechanism for metallothionein induction by metal cations

Cellular effects of exposure to the divalent metal cations and roles of particular resistance mechanism of the metallothionein expression, and expression of stress protein GroEL is shown in the **Figure 3**. GroEL forms essential component which reduces heavy metal stress [22]. The MTs is a cysteine rich protein which binds with the metals and thereby detoxified through reducing its presense [23]. The heavy metals enter the cells by the transport of the proteins which binds the metals. This metals could further binds SH groups with repressor protein of smtB. These attached metals further alter conforms the repressor protein and then released it. These process permits RNA polymerase to initiate the transcription from smtA, resulted the metallothionein expression.

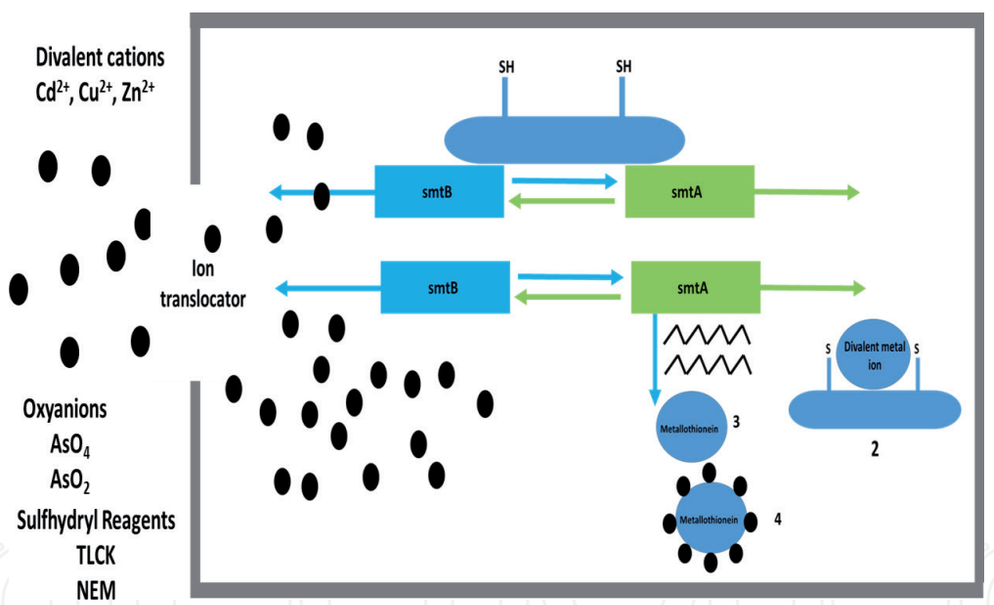

Figure 3.
Proposed mechanism for metallothionein induction by metal cations and arsenic oxyanions. (1) divalent metal cation challenge from the environment, (2) sulfhydryl (-SH) modification by metal or arsenate binding, (3) SmtA expression producing metallothionein proteins, (4) metal sequestration and detoxification by metallothionein proteins.

7. Plant metallothioneins

After the Phytochelatin discovery, as a key metal ligand essential for the plant tolerance to Cadmium, a MT protein was identified in the wheat, and numerous MT genes was isolated from the plants. The plant Class II MT proteins are further classified based upon the amino acid sequence. In the year 1993, Robinson and his co-workers identified the two plant based MT which was based on cysteine residue positions in the predicted proteins. Since then, number of characterized plant MT genes has been increased drastically, as many do not conform to these 2 groups, some additional categories are added [24, 25].

The majority of the plant MT genes are identified in angiosperms. Numerous species, like *Arabidopsis*, sugarcane and rice contain some genes encoding all 4 MTs types. This shows that the evolution of 4 plant MT types predates the separation of the monocots and dicots, and are likely that the major flowering plants also contains 4 different MT types. The presence of 4 MTs types in the plants with a distinct arrangement of the cysteines contrasts with situation in animals. For an example, the 4 mouse MTs all contain the same conserved cysteines, although they do differ in tissue expression [17]. The diversity of plant MT gene family suggests that these might differ not only in the sequence but also in the function. There is only little information about the MT genes in the non-flowering plant species. However, the genes encoding the Type3 MTs are cloned from different gymnosperms. One of the MT-encoding gene are isolated from a brown alga, *Fucus vesiculocus*, [26]. This MT do not fit readily into any of the 4 plant types described above but, primarily relies on basis of cysteine residues, is equally similar to the Arabidopsis MT1a and an oyster MT.

8. Expression pattern under influence of various factors

8.1 Metal ions

As homologs of animal MTs, it would be reasonable to assume that the MTs in plants are related to metal metabolism and detoxification of excess metal ions. Indeed, the expression of both Ec proteins and several class I MT genes vary under metal treatment. The wali l (type 2) in wheat was originally isolated as an aluminum-induced gene. Its expression is also increased by treatment with other toxic metal ions like Cd, Fe, Zn, Cu, Ga, In and La.

In rice, the transcript level of RgMT (type 1) was elevated by 250 μM $CuCl_2$, in suspension-cultured cells [27, 28], while 100 μM $CuSO_4$ increased ricMT (type 4) transcripts in both shoots and roots of seedlings. This expression-enhancing effect of Cu^{2+} are been reported in the type 2 MT genes from *Arabidapsis* and tobacco, and in type 3 MT from *Arabidopsis*. In contrast, copper ion are shown to have a suppressive effect on type 2 MT in soybean and *Vicia faba*; in the copper-tolerant plant *Mimulus guttatus*, transcription of the type l MT was inhibited by 5 μM CuS04, as well as by 5 μM $CdSO_4$ and 15 μM $ZnSO_4$. In addition, the mRNA Ievel of ricMT in the root of rice seedlings was reduced by 100 μM aqueous solutions of $ZnSO_4$, $CdCl_2$, $FeCl_3$, Pb $(Ac)_2$ and $AlC1_3$, respectively.

8.2 Senescence

Coupe *et al*. [29] isolated the type 2 MT gene JET12 from abscission zones of the ethylene-treated leaves of *Sambucus nigra* L. Northern analysis reported that JET12 gene was expressed only in senescent leaves, and in the ethylene-treated abscission zone.

Similarly, the type 3 MT LSC54 in rape (*Brassica napus*) was expressed during leaf senescence, and as senescence progressed, the mRNA level of LSC54 increased. Yu *et al*. [28] showed that the expression of rgMT (type 1) increased as young leaves progressed to senescence. These findings suggest that these MT genes may play a key role in senescence-associated metabolism of plants.

8.3 Other stresses

Considerable effort are made to clarify the relationships between plant MTs and environmental factors other than metal ions. The expression of type 2 MT seems to

be easily affected by stresses- For example; the MT in tobacco was strongly induced by tobacco mosaic virus and wounding [30] and expression of MT in *Vicia faba* was reduced when plants were treated with salt, cold, salicylic acid or UV-light. Furthermore, transcripts of OsMT-2 in rice can be induced by sucrose sta, heat shock and ABA and the expression of wali l in wheat was increased by low-nutrient conditions and wounding.

Regarding type I MT, the mRNA level of rgMT in rice increased under treatment with heat shock, a toxicant (DMSO) and starvation, and decreased in presence of abscisic acid.

9. Fungal metallothioneins

Fungal MTs, study are more sporadic and are described usually as copper binding proteins. Still, few are characterized functionally in the positions of metal organization in chemical properties or complementation tests in mutants of metal sensitive yeasts. Reputed MTs are mostly designated within collections of ESTs (Expressed Sequence Tag) based on sequence resemblance. These are occasion for the MT like arrangements that are present in the mycorrhizea, specifically in *Pisolithus tinctorius* (ectomycorrhizal fungi), the ericoid fungus (*Oidiodendron maius)* and the arbuscular mycorrhizal fungi (*Gigaspora rosea*, *Gigaspora margarita* and *Glomus intraradices)* [31, 32].

The *P. involutus* gene (*Pimt1*) codes for short MT (34 amino acids) and contains domain bearing classical C–X–C motifs, compared with longer and canonical MTs that usually have 2 Cys-rich domains. These are found in the other fungal MTs too; it appears to be that length is not critical required for binding of metal, as even some shorter protein of MT has proved to efficiently chelate the metal ions [33].

Heterologous expression systems for demonstrating unambiguously that *Pimt1* gene product can sequester metal ions, therefore conferring *in vivo* protection against the metals, in particular with cadmium and copper [34]. The functional complementation assays was done using 3 distinct mutants of metal-hypersensitive yeast. Similar approach was done for the characterization of 2 MT- encoding genes from the endomycorrhizal fungi [31]. It explains in what way Genome Omission Project for *Saccharomyces* offered materials towards the efficient genomics researches in area of the metal forbearance. Availability of abundant metal sensitive strains lets *in vivo* segmentation of the metal protection mechanism and ultimately lead for identifying specific molecular roles that are played by the DNA sequence of interest.

There are more evidence for MTs role or MT-like proteins in the mutualistic and the pathogenic interactions in between fungi and plants. The genes of fungal MT are mostly regulated transcriptionally in the life stages mainly, during the colonization of plant. The first report was in 1995, when the 2 genes similar to MTs were expressed uniquely during appressorium formation by of conidia in *Colletotrichum gloeosporioides* which are induced by the host surface wax. The 2 putative MT genes from biotrophic pathogen (*Uromyces fabae)* are upregulated strongly in the parasitic mycelium that are colonizing tissues of leaves [35]. So far as the mycorrhizal fungi are concerned, the *Pimt1* was found in macroarray experiments as upregulated in ectomycorrhizal tissues compared with the saprotroph growth condition. In contrast, MTs from arbuscular mycorrhizal fungi appear to be downregulated when the fungus colonizes the root tissues. It is worth noting that plant MTs are also upregulated in ectomycorrhizal associations of *Betula pendula* with *Eucalyptus globulus* and *P. involutus* with *P. tinctorius*, respectively. To support further MTs role in the plant–fungus interaction, Tucker *and* his co-workers described one unusual MT-like protein with 22 amino acids long with 6 cysteines in *Magnaporthae grisea*

(fungal pathogen), that showed high affinity to zinc. Gene has no effect in the metal tolerance and it is shown to confer the pathogenicity, through playing role in the biochemical differentiation of appressorium cell wall [33].

10. Cyanobacterial metallothionein

Numerous mechanisms of metal resistance exist in cyanobacteria. The first mechanism involves extracellular binding. Cells might synthesize and release some organic materials that could chelate the metals and reduce their bioavailability or metal ions might bound to outer cell surface [36]. These complex forms are not readily transported into cell due to its complexity and structure. Secondly, cells could increase excretion rate of some metal ions using the energy-driven efflux pumps [37]. The sequestration of internal metal, a 3rd resistance mechanism, is a key mechanisms through which the bacteria combats the exposure of the heavy metal and their subsequent accumulation. In cyanobacteria, metal ion sequestration inside the cell are performed by the Class II MTs.

The Class II MTs are a cysteine-rich, thiol-containing and are metal-binding proteins that can sequester the metals, thereby stopping the buildup of potentially some deadly free metals in cell. Metal binding happens by interactions of the metals with thiol clusters of the cysteine remainders. MTs gene was organized as operon termed as smt locus, that contains together the metallothionein protein (smtA) and the regulatory protein, repressor (smtB) genes. The SmtBs, are the managing repressor of the countenance from smtA promoter- operator province. The MTs countenance, from gene to the efficient protein, was encouraged through metals and regulation of the transcript to mRNA is based on the interface among the metals and repressor protein that regulates transcript, over by the interface by a thiol clusters that are contemporary on the repressor type of protein [38, 39].

11. Mammalian metallothionein

The MT genes were readily induced by several toxicologic and physiologic stimuli. Since the MT cysteines are conserved across the species, it is suspected that the cysteines are essential for the function and the MTs are required for the life. In attempts for determining the MT function(s), researches were carried out with 4 different experimental paradigms: (i) the animals were injected with chemicals that induce MT; (ii) the cells adapted to grow and survive in higher MT concentrations - that induces toxicants; (iii) the cells are transfected with the MT gene; and (iv) the MT-null mice and the MT transgenic. Very often, the reports from the researches with the first 3 approaches had indicated several purposes of MT in the cell biology is as follows: the MT (a) is “warehouse” for the “Zn” (b) the free radical hunter (c) protect from cadmium harmfulness. However, researches with MT transgenic with the null rats did not toughly reinforced initial 2 proposed purposes then they sturdily sustenance its purpose in the protection in contradiction of the cadmium harmfulness. Recurrent cadmium management to the MT null rats resulted in the nephron harmfulness at the 1/10 of the quantity that products the toxicity in the control rats. Researches in human being showed as 7% of overall populations had some renal malfunction by cadmium exposures. Consequently, if the human being did not consume the MT, “standard” exposure of cadmium will be toxic to human beings [17]. Thus, it appears that during evolution, the MT ability to protect against the Cd toxicity would had taken a chief role for the life processes maintenance, as compared with its other proposed functions (*i.e.* storehouse for zinc and free radical scavenger) [40, 41].

12. Studies conducted at Tamil Nadu agricultural university

Expression of metallothionein 3-like protein mRNA in the sorghum cultivars under the chromium (VI) stress was estimated [20]. The *MT3* gene expression in roots after imposition of 100 μM Cr (VI) for five days in fifteen days old seedlings of 2 sorghum cultivars, *viz.* K 10 and CO - 27 (susceptible and tolerant respectively, based on earlier studies on growth and antioxidant polymerase chain reaction (RT–PCR), as described by Kaplana *et al*. [42]. After the reports attained it is shown as the sorghum variety CO - 27 pretreated with the Chromium (VI) represented some great strength band which matches the genetic factor of attention. These suggestions can be increased MT transcript degrees under the chromium strain, predominantly in the forbearing variety. Now it is probable that Reactive Oxygen Species (ROS) and hydrogen peroxide that are produced under the chromium stress acts as signal for inducing MT mRNA transcription. The phytochelatin functions in regulation of essential metals and in the detoxification of several toxic metals. Distinct absence of the phytochelatin are shown in the plants under the Cr (VI) stress. These suggested that there can be enhanced role for the MTs in plants under the chromium stress.

13. Limitations

Study on the MT structure of plant and their function are impaired by difficulties that are encountered in the purification of this proteins since the innate bases, mostly because of intrinsic variability of the cysteine rich polypeptides with the occurrence of oxygen. Very few MTs are recuperated from the plant cells, and are uniform some as integral, noncleaved collections of metal [6]. The heterologous countenance approaches stunned this complications, but the early results exhibited as the pea MTs produced in *Escherichia coli* as innate protein were likewise vulnerable to proteolysis. Alternative strategies for producing the plant MTs as the fusion proteins generated some intact proteins that retained their metal binding and their antigenic features [43]. Inappropriately, the cleavage and the retrieval of MTs portion were not made and so the description of the consistent collections of metals were not shown. But, GST founded expression system are functional to various inborn MT forms allows amalgamation of huge quantity of the identical metal MT provisions. The investigation of this developments by the spectrometric and the spectroscopic systems have given some unparalleled data about the MT collective stoichiometric and the manageable performance of MTs [44].

14. Conclusion

MTs are stress related protein and might be involved in essential metal homeostasis and detoxification of excess metals during organism's developmental process. The MT genes are readily induced by several toxicologic and physiologic stimuli. Due to the cysteines in MT are absolutely conserved across the species, it is suspected that cysteines are essential for the function and MT is required for the life. A clear role for MTs is yet to be established, although they are certainly thought to play a key role in the metal metabolism. MTs may function as the antioxidants and another possibility is the role in repair of the plasma membrane. Although MTs are expressed ubiquitously and conserved in living organisms, determining their function remains a future challenge. So further research work is necessary to better

understand MT-metal interaction, the mechanism of MT expression and molecular transformation of novel MT genes to plant and microbial system for increasing heavy metal detoxification in the environment.

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