Balance between metallothionein and metal response element binding transcription factor 1 is mediated by zinc ions (Review)

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Abstract. Metal ion homeostasis and heavy metal detoxification systems are regulated by certain genes associated with metal ion transport. Metallothionein (MT) and metal response element binding transcription factor 1 (MTF-1) are important regulatory proteins involved in the mediation of intracellular metal ion balance. Differences in the zinc-binding affinities of the zinc fingers of MTF-1 and the α- and β-domains of MT facilitate their regulation of Zn^{2+} concentration. Alterations in the intracellular concentration of Zn^{2+} influence the MTF-1 zinc finger number, and MTF-1 containing certain zinc finger numbers regulates the expression of corresponding target genes. The present review evaluates the association between zinc finger number in MTF-1 protein, MTF-1 target genes and the mechanism underlying MT regulation of the zinc finger number in MTF-1.

Contents

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1. Introduction

Cells adjust their gene expression profile in order to rapidly adapt to various forms of stress. Therefore, stress-responsive transcription factors are mainly regulated at the post-translational level by modulating activity, stability or subcellular localization (1). Certain transcription factors predominantly localize to the cytoplasm and translocate to the nucleus when required (1). The zinc ion (Zn^{2+}) is an important metal ion involved in the regulation of transcription factors. Zn^{2+} and other essential metal ions have diverse roles in numerous biological processes by functioning as structural components of proteins, essential co-factors in enzymes and/or modulators of signal transduction cascades within cells (2). Furthermore, all metal ions are cytotoxic at high intracellular concentrations.

Metallothionein (MT) is a small, cysteine-rich protein, which binds and exchanges specific metal ions, particularly Zn^{2+} (3). The binding affinity of MT varies between metals, with Cu^{+} having the greatest stability constant (10^{19}-10^{17}), followed by Cd^{2+} (10^{17}-10^{15}) and Zn^{2+} (10^{14}-10^{11}). Up to 18 metals are able to associate with MT, but Zn^{2+} is only able to be displaced by Cu^{+}, Cd^{2+}, Pb^{2+}, Ag^{+}, Hg^{2+} and Bi^{2+} (4). MT comprises two subunits: The more stable α-domain (C-terminal), which incorporates four divalent metal atoms, and the β-domain (N-terminal), which contains only three divalent metal ions and is more reactive (5). MT exchange ability depends upon the metal species present, and the majority of MTs exist in zinc form or as mixed-metal proteins *in vivo* (6).

Numerous ions activate the promoter of the MT gene via metal response elements (MREs), but only Zn is specific for the binding and activation of MRE-binding transcription factor-1 (MTF-1) (7). MTF-1 is a zinc finger transcription factor, which regulates metal-responsive gene expression (8). The MTF-1 protein contains a six-Cys_2His_2 zinc finger DNA-binding domain, three transcriptional activation domains as well as putative nuclear exclusion sequences (NES) and localization sequences (NLS) (8).

2. Metallothionein is regulated by metal ions and oxidant stress

A previous study demonstrated that *in vivo* MTs exist mainly in Zn form or as mixed-metal proteins (6). The zinc ion of the

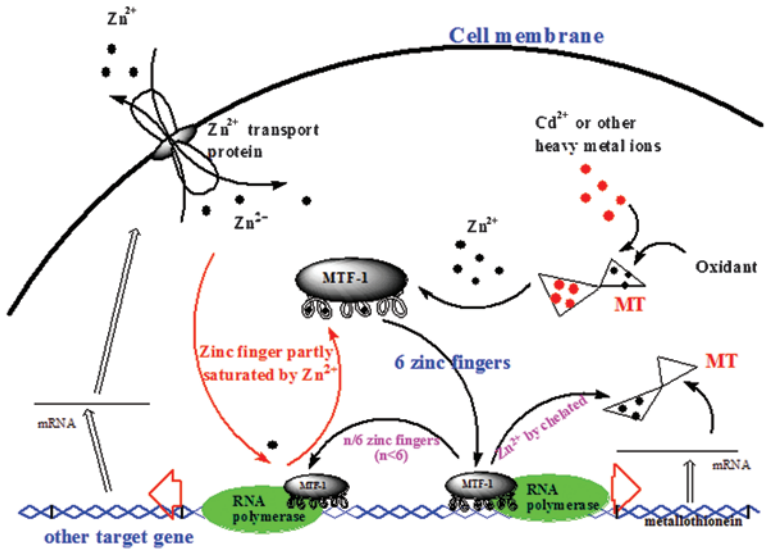

Figure 1. Scheme of mammalian MTF-1 regulation. Under normal conditions there is a dynamic balance between MT and MTF-1 in order to regulate the transport of certain metal ions via MTF-1 inducing target gene expression. MTF-1 is able to be activated directly by zinc or indirectly by the release of zinc from MTs following cadmium load or oxidative stress. MTF-1 containing six zinc fingers subsequently promotes transcription of the MT gene. Superabundant MT protein captures Zn^{2+} from MTF-1, and MTF-1 interacts with other transcription factors and coactivators to drive the expression of additional target genes due to the change in zinc finger number. As a result, normal conditions are restored to the cell as the genes expressed eliminate unfavorable factors. MT, metallothionein; MTF-1, metal response element binding transcription factor 1; mRNA, messenger RNA.

Zn_7-MT complex is able to be replaced by numerous heavy metal ions, including copper and cadmium, when these are present in the cell. Furthermore, the zinc ion is released from the Zn_7-MT complex following exposure to reactive oxygen species. The free zinc ion, from the Zn_7-MT complex or another metal-MT complex, is chelated by the six zinc fingers of MTF-1, which is activated by binding of Zn^{2+} to the six zinc fingers (9). MTF-1 induces expression of the MT gene via binding DNA and other transcription factors and the novel MT subsequently combines redundant free Zn^{2+} or binds Zn^{2+} from MTF-1 in the cytoplasm (9). A proportion of the zinc ions bound to MTF-1 are captured by MT due to the higher stability constant of MT with zinc ions, compared with those for partial chelation of the six zinc fingers of MTF-1 (9). MT expression is reduced or halted entirely following the release of zinc from MTF-1 (10).

3. Regulatory effect of MTF-1 on target genes

The DNA-binding domain of MTF-1 comprises six zinc fingers that mediate binding to the MRE DNA motif, which contains the core consensus sequence 'TGCRCNC' (8). MREs are frequently located in the promoter/enhancer regions of MTF-1 target genes in multiple copies. The MT genes are the most comprehensively studied of these target genes (11). A study to identify MTF-1 target genes other than MT genes has been performed in human, mouse and *Drosophila* systems, and ~27 genes were identified (9). As exhibited in Table I, 13 target genes are involved in metal ion metabolism, which regulate cellular Zn^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+} and Li^{+} homeostasis, respectively (12-21). Mammalian target genes with a role in metal homeostasis that are induced following zinc load include solute carrier family 30 member 1 (Slc30a1) and Slc30a2. These encode the zinc exporters ZnT-1 and ZnT-2, respectively (14,22). In addition, MTF-1 suppresses the transcription of Slc39a10 (13), which was demonstrated to encode the zinc importer Zip10 (23). MTF-1 may select the appropriate type of MRE depending on whether there is a high or low intracellular concentration of Zn^{2+} (24,25). The results of the studies described above also indicated that the transport of zinc ions into a cell was prevented when all six zinc fingers of MTF-1 chelated zinc ions. In order to elucidate why MTF-1 was able to regulate other target genes, a hypothesis was developed, based on the association between protein structure and function, suggesting that MTF-1 may regulate target genes other than MT when the six zinc fingers of MTF-1 were not saturated by zinc ions. The chelation of zinc fingers in MTF-1 influences the protein structure of MTF-1 and therefore mediates the selection of various MREs from the same or different promoters.

Not only the zinc ion is able to influence the protein structure of MTF-1. Recently, using genome-wide mapping of MTF-1 binding under Cu and Cd metal stresses, Sims *et al* (26) demonstrated that MTF-1 DNA-binding site specificity depended on the specific nature of the metal, and also discovered that the type of binding site which exhibited affinity for the metal ion was an important factor in the induction of metal-specific transcription activation. Similarly, the analagous domain of *Drosophila* MTF-1 activated differential target genes under the opposing conditions of copper load and copper starvation (27). Other regions of MTF-1 also influenced the specific binding of MTF-1 to DNA sequences; for example, an allosteric change of the conserved C-terminus of the cysteine-rich domain in *Drosophila* MTF-1 required cadmium binding (28). Certain transcription factors, including nuclear hormone receptors, have also been shown to exhibit binding-site diversity that dictates their specific transcriptional activation and interactions with ligands and co-factors (29).

Previous studies on the variations in the sequence affinity of MTF-1 suggested that at least a proportion of its zinc

Table I. Metal ion homeostasis target genes of mammalian and *Drosophila* MTF-1.

Metal ion type	Gene/protein	Organism	Regulation via MTF-1	Function	Ref.
Heavy metal ion	Metallothionein	Mouse/human/*Drosophila*	Basal and metal induced	Metal ion homeostasis, heavy metal ion detoxification	(12)
Zn^{2+}	Slc30a1/zinc transporter-1	Mouse	Basal and Zn/Cd induced	Zinc ion transport, calcium ion import	(13)
	Slc30a2/zinc transporter-2	Mouse	Zn induced	Zinc ion transport	(14)
	Slc39a10/zinc transporter Zip10	Mouse	Basal and Zn/Cd induced	Zinc ion transport	(13)
	ZnT35C/zinc transporter 35c	*Drosophila*	Basal and Zn/Cd induced	Zinc ion transport	(15)
	Slc39a11/ Zip11	Mouse/human	Basal	Zinc importer	(16)
Cu^{2+}	PRNP/PRNP	Human	Cu induced	Cellular copper ion homeostasis	(17)
	CG10505	*Drosophila*	Zn/Cd/Cu induced	Copper transport, cadmium transport	(15)
	CG1886/ATP7	*Drosophila*	Cu induced	Copper ion export	(18)
	CG7459/Ctr1B	*Drosophila*	Basal and low Cu induced	Copper ion transport	(19)
Cd^{2+}	See metallothionein See Slc30a1 See CG10505				
Fe^{2+}	Slc40a1/ferroportin-1	Mouse	Zn/Cd induced	Iron ion transport	(20)
	Fer2LCH, Fer1HCH/ferritin	*Drosophila*	Zn/Cd/Cu induced	Cellular iron ion homeostasis	(15)
Li^{+}	C-EBPα/CCAAT-enhancer-binding protein-α	Mouse	Basal and Zn induced	Lithium ion homeostasis	(21)

MTF-1, metal response element binding transcription factor 1; PRNP, major prion protein; Ctr1B, copper transporter 1B.

fingers were involved in sensing cellular zinc levels. These studies were based on the fact that MTF-1 was activated by elevated levels of zinc in cell-free DNA binding studies, and the observed variations in zinc-binding affinity of the zinc fingers (30-32). To date, studies aiming to elucidate the role of individual zinc fingers in zinc sensing have yielded ambiguous results (4). However, there is evidence that zinc fingers 1-4 comprise the core DNA-binding domain and that zinc fingers five and six may act as zinc sensors involved in the mediation of metal-responsive transcription (33).

In a previous study by our group, it was demonstrated that in *Tetrahymena thermophila (T. thermophila),* MTT1 and MTT2 were shown to have differing primary structural characteristics, and to perform different functions in cells. MTT1 was shown to have a role in the detoxification of heavy metal ions, and MTT2 may be involved in the homeostasis of copper ions (34-36). Subsequently, it was reported that MTT1 and MTT2 had distinct transcriptional mechanisms, regulated by different MTF-1s, and that three zinc finger proteins, which were corresponded to MT transcription-associated factors in the *T. thermophila* genome, contained varying numbers of zinc finger proteins (37). Number-specific zinc finger proteins had metal-specific conformations, recognized specific MRE sequences and promoted transcription of specific, corresponding genes (37). Based on the results of the aforementioned study, the number of zinc fingers in MTF-1 which are zinc ion saturated in this protein is associated with the mediation of MTF-1 target gene transcription.

4. Balance between metallothionein and MTF-1

Zinc has a wide repertoire of known biological functions, including as a cofactor in numerous enzymes and DNA-interacting proteins (38,39). Zinc deficiency, as well as excess, has been shown to be deleterious for cells (40), prompting the requirement for tight regulation of its cellular concentration (41). MT and MTF-1 form a pair of powerful regulators of cellular zinc ion concentration. Under normal conditions, a chemical equilibrium, directed by cellular zinc ion concentration, is generated between MT and MTF-1. This equilibrium is disturbed when the cell is exposed to certain stressors, including heavy metal ions and oxidants. A study also indicated that MTF-1 may be stimulated by the degradation of zinc-saturated MT (42).

MTF-1 contains a metalloregulatory DNA-binding domain consisting of six Cys_2His_2 zinc fingers, which is thought to bind zinc with affinities in the nanomolar to sub-micromolar range (31). The order of zinc affinities of the six zinc fingers of MTF-1 (from highest to lowest) was demonstrated to be: 4>2>5>6>3>1 (31). Nuclear magnetic resonance analysis of zinc finger proteins from the MTF-1 DNA-binding domain indicated that the zinc-binding affinities of all six MTF-1 zinc fingers were within (~10-50)-fold of each other (32). The relative zinc-binding affinities of the seven sites in the MT α- and β-domains were determined via direct competition for Zn^{2+}, and the results demonstrated that Zn^{2+} binds the β-domain with a greater affinity than the α-domain (43). The presence of zinc affinity gradients in MT and MTF-1 provide a precise basis for the adjustment of the balance between MT and MTF-1 concentration and allows MTF-1 to regulate transcription of genes associated with zinc and other metal ion transport.

5. Conclusions and perspectives

In conclusion, the results discussed in the present review provided novel insights into the dynamic balance of MT and MTF-1 concentration, and supported novel hypotheses for the mechanisms underlying the collaborative regulation of certain genes linked with metal ion transport by MT and MTF-1. As summarized in Fig. 1, MTF-1 regulates MT transcription in order to respond to other heavy metal ions or oxidative stress, and modulates the expression of other metal transport proteins via a subtle collaboration with MT. An association between MTF-1 target genes and the zinc finger number in MTF-1 proteins has been indicated. However, the mechanism underlying the regulation of other candidate target genes by MTF-1 binding different amounts of Zn^{2+} remains elusive. Further study is therefore required in order to elucidate the potential of MTF-1 in the regulation of transcription of other target genes by altering the zinc finger number. An evaluation of the specific association between MTF-1 and its target genes following confirmation of the number of zinc ions bound to the MTF-1 protein is required to further elucidate the mechanisms underlying this complex interaction. In conclusion, there is an interesting balance between metallothionein and metal response element binding transcription factor 1 is mediated by zinc ions. However, the mechanisms of balance mediated by zinc finger motif of MTF-1 remains to be elucidated in future studies.

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References

1. Lindert U, Cramer M, Meuli M, Georgiev O and Schaffner W: Metal-responsive transcription factor 1 (MTF-1) activity is regulated by a nonconventional nuclear localization signal and a metal-responsive transactivation domain. Mol Cell Biol 29: 6283-6293, 2009.
2. Kambe T, Weaver BP and Andrews GK: The genetics of essential metal homeostasis during development. Genesis 46: 214-228, 2008.
3. Davis SR and Cousins RJ: Metallothionein expression in animals: a physiological perspective on function. J Nutr 130: 1085-1088, 2000.
4. Coyle P, Philcox JC, Carey LC and Rofe AM: Metallothionein: the multipurpose protein. Cell Mol Life Sci 59: 627-647, 2002.
5. Chen P, Munoz A, Nettesheim D, Shaw CF III and Petering DH: Stoichiometry and cluster specificity of copper binding to metallothionein: homogeneous metal clusters. Biochem J 317 (Pt 2): 395-402, 1996.
6. Orlowski C and Piotrowski JK: Metal composition of human hepatic and renal metallothionein. Biol Trace Elem Res 65: 133-141, 1998.
7. Andrews GK: Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochem Pharmacol 59: 95-104, 2000.
8. Laity JH and Andrews GK: Understanding the mechanisms of zinc-sensing by metal-response element binding transcription factor-1 (MTF-1). Arch Biochem Biophys 463: 201-210, 2007.
9. Günther V, Lindert U and Schaffner W: The taste of heavy metals: gene regulation by MTF-1. Biochim Biophys Acta 1823: 1416-1425, 2012.

10. Zhang B, Georgiev O, Hagmann M, *et al*: Activity of metal-responsive transcription factor 1 by toxic heavy metals and H_2O_2 in vitro is modulated by metallothionein. Mol Cell Biol 23: 8471-8485, 2003.
11. Günther V, Davis AM, Georgiev O and Schaffner W: A conserved cysteine cluster, essential for transcriptional activity, mediates homodimerization of human metal-responsive transcription factor-1 (MTF-1). Biochim Biophys Acta 1823: 476-483, 2012.
12. Radtke F, Heuchel R, Georgiev O, *et al*: Cloned transcription factor MTF-1 activates the mouse metallothionein I promoter. EMBO J 12: 1355-1362, 1993.
13. Wimmer U, Wang Y, Georgiev O and Schaffner W: Two major branches of anti-cadmium defense in the mouse: MTF-1/metallothioneins and glutathione. Nucleic Acids Res 33: 5715-5727, 2005.
14. Guo L, Lichten LA, Ryu MS, Liuzzi JP, Wang F and Cousins RJ: STAT5-glucocorticoid receptor interaction and MTF-1 regulate the expression of ZnT2 (Slc30a2) in pancreatic acinar cells. Proc Natl Acad Sci USA 107: 2818-2823, 2010.
15. Yepiskoposyan H, Egli D, Fergestad T, *et al*: Transcriptome response to heavy metal stress in *Drosophila* reveals a new zinc transporter that confers resistance to zinc. Nucleic Acids Res 34: 4866-4877, 2006.
16. Yu Y, Wu A, Zhang Z, *et al*: Characterization of the GufA subfamily member SLC39A11/Zip11 as a zinc transporter. J Nutr Biochem 24: 1697-1708, 2013.
17. Bellingham SA, Coleman LA, Masters CL, Camakaris J and Hill AF: Regulation of prion gene expression by transcription factors SP1 and metal transcription factor-1. J Biol Chem 284: 1291-1301, 2009.
18. Burke R, Commons E and Camakaris J: Expression and localisation of the essential copper transporter DmATP7 in *Drosophila* neuronal and intestinal tissues. Int J Biochem Cell Biol 40: 1850-1860, 2008.
19. Selvaraj A, Balamurugan K, Yepiskoposyan H, *et al*: Metal-responsive transcription factor (MTF-1) handles both extremes, copper load and copper starvation, by activating different genes. Genes Dev 19: 891-896, 2005.
20. Troadec MB, Ward DM, Lo E, Kaplan J and De Domenico I: Induction of FPN1 transcription by MTF-1 reveals a role for ferroportin in transition metal efflux. Blood 116: 4657-4664, 2010.
21. Lichtlen P, Wang Y, Belser T, *et al*: Target gene search for the metal-responsive transcription factor MTF-1. Nucleic Acids Res 29: 1514-1523, 2001.
22. Langmade SJ, Ravindra R, Daniels PJ and Andrews GK: The transcription factor MTF-1 mediates metal regulation of the mouse ZnT1 gene. J Biol Chem 275: 34803-34809, 2000.
23. Kaler P and Prasad R: Molecular cloning and functional characterization of novel zinc transporter rZip10 (Slc39a10) involved in zinc uptake across rat renal brush-border membrane. Am J Physiol Renal Physiol 292: F217-F229, 2007.
24. Wang Y, Lorenzi I, Georgiev O and Schaffner W: Metal-responsive transcription factor-1 (MTF-1) selects different types of metal response elements at low vs. high zinc concentration. Biol Chem 385: 623-632, 2004.
25. Devergnas S, Chimienti F, Naud N, *et al*: Differential regulation of zinc efflux transporters ZnT-1, ZnT-5 and ZnT-7 gene expression by zinc levels: a real-time RT-PCR study. Biochem Pharmacol 68: 699-709, 2004.
26. Sims HI, Chirn GW and Marr MT II: Single nucleotide in the MTF-1 binding site can determine metal-specific transcription activation. Proc Natl Acad Sci USA 109: 16516-16521, 2012.
27. Günther V, Waldvogel D, Nosswitz M, Georgiev O and Schaffner W: Dissection of Drosophila MTF-1 reveals a domain for differential target gene activation upon copper overload vs. copper starvation. Int J Biochem Cell Biol 44: 404-411, 2012.
28. Marr SK, Pennington KL and Marr MT: Efficient metal-specific transcription activation by *Drosophila* MTF-1 requires conserved cysteine residues in the carboxy-terminal domain. Biochim Biophys Acta 1819: 902-912, 2012.
29. Meijsing SH, Pufall MA, So AY, Bates DL, Chen L and Yamamoto KR: DNA binding site sequence directs glucocorticoid receptor structure and activity. Science 324: 407-410, 2009.
30. Heuchel R, Radtke F, Georgiev O, Stark G, Aguet M and Schaffner W: The transcription factor MTF-1 is essential for basal and heavy metal-induced metallothionein gene expression. EMBO J 13: 2870-2875, 1994.
31. Guerrerio AL and Berg JM: Metal ion affinities of the zinc finger domains of the metal responsive element-binding transcription factor-1 (MTF1). Biochemistry 43: 5437-5444, 2004.
32. Potter BM, Feng LS, Parasuram P, *et al*: The six zinc fingers of metal-responsive element binding transcription factor-1 form stable and quasi-ordered structures with relatively small differences in zinc affinities. J Biol Chem 280: 28529-28540, 2005.
33. Chen X, Chu M and Giedroc DP: MRE-Binding transcription factor-1: weak zinc-binding finger domains 5 and 6 modulate the structure, affinity, and specificity of the metal-response element complex. Biochemistry 38: 12915-12925, 1999.
34. Wang Q, Xu J, Zhu Y, Chai B, Liang A and Wang W: Lanthanum(III) impacts on metallothionein MTT1 and MTT2 from *Tetrahymena thermophila*. Biol Trace Elem Res 143: 1808-1818, 2011.
35. Wang Q, Xu J, Chai B, Liang A and Wang W: Functional comparison of metallothioneins MTT1 and MTT2 from *Tetrahymena thermophila*. Arch Biochem Biophys 509: 170-176, 2011.
36. Wang Q, Xu J, Chai B, Liang A and Wang W: Expression of Metallothionein MTT1 and MTT2 from *Tetrahymena thermophila* exposed to SO_2, NaCl and Tris-HCl. Asian J Chem 24: 705-708, 2012.
37. Zhang P, Xu J, Lu J, Liang A and Wang W: Different response of two metallothionein subfamilies exposed to chromium (vi) in Tetrahymena thermophila. Fresen Environ Bull 22: 1924-1928, 2013.
38. Stefanidou M, Maravelias C, Dona A and Spiliopoulou C: Zinc: a multipurpose trace element. Arch Toxicol 80: 1-9, 2006.
39. Maret W and Li Y: Coordination dynamics of zinc in proteins. Chem Rev 109: 4682-4707, 2009.
40. Wright RO and Baccarelli A: Metals and neurotoxicology. J Nutr 137: 2809-2813, 2007.
41. Kukic I, Lee JK, Coblentz J, Kelleher SL and Kiselyov K: Zinc-dependent lysosomal enlargement in TRPML1-deficient cells involves MTF-1 transcription factor and ZnT4 (Slc30a4) transporter. Biochem J 451: 155-163, 2013.
42. Kimura T, Okumura F, Oguro I, *et al*: Metal response element-binding transcription factor-1 is activated by degradation of metallothionein. J Health Sci 55: 72-76, 2009.
43. Summers KL, Sutherland DE and Stillman MJ: Single-domain metallothioneins: evidence of the onset of clustered metal binding domains in Zn-rhMT 1a. Biochemistry 52: 2461-2471, 2013.