— Minireview —

Transcriptional Regulation of the Metallothionein Genes

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(Received March 15, 2004)

The classical mammalian metallothioneins (MT-I/II) and the other subfamilies (MT-III/IV) both bind heavy metals by the well-conserved cysteine cluster structure, but their modes of expression differ from each other. MT-I/II is ubiquitously induced by heavy metals, but MT-III/IV is expressed in restricted tissues without obvious dependency on heavy metals. To understand the heavy metal dependent transcriptional activation of the MT-I/II genes, the mechanism of the heavy metal response of a pivotal transcription factor, MTF-1, has been extensively studied. On the other hand, in the case of MT-III, the mechanism of gene suppression has been investigated to clarify the basis of its tissue specific expression. In this review, recent progress in understanding the transcriptional regulation of these MT subfamilies is described.

Key words —— metallothionein, transcription, heavy metal, MTF-1, metal responsive element

HEAVY-METAL DEPENDENT TRANSCRIPTIONAL ACTIVATION OF THE METALLOTHIONEINS (MT-I/II) GENES

The hallmark of classical metallothioneins [MTs (MT-I/II)] is their ubiquitous induction by various heavy metals including cadmium (Cd) and zinc (Zn). This induction is mainly controlled at the level of transcription, which is regulated *via* an enhancer sequence designated metal responsive element (MRE), and a heavy metal dependent MRE-binding factor, MTF-1.[1,2] MTF-1 has well-conserved protein structure from insects to humans,[3–6] which contains six tandem repeats of the C_2H_2 type zinc finger motif in its N-terminal half, and three transcriptional activation domains in its C-terminal half, but there are no apparent sensing modules for heavy metals such as the metallothionein-like cysteine cluster structure discovered in ACE1,[7] a transcription factor for the yeast MT gene.

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Response of MTF-1 to Heavy Metals

MTF-1 is essential for the heavy-metal dependent transcriptional activation of the MT-I/II genes. This is evidenced by the fact that mouse embryonic stem cells lacking both MTF-1 alleles cannot induce MT-I/II by any heavy metals so far investigated.[8] Contrary to this result *in vivo*, Zn is the only heavy metal able to induce MTF-1 binding to MRE *in vitro*.[9–11] This conflict between *in vivo* and *in vitro* results has led to the idea that Zn is the second messenger of the heavy metal signals, and MTF-1 is a sensor protein responsive to an increase in the intracellular concentration of free Zn.[12] Such an increase in the Zn-concentration is thought to originate from Zn-release from the hypothetical Zn-pool, which is prompted by other heavy metals. In this regard, Daniels *et al*. have reported that a reporter assay system in yeast cells, which is driven by MTF-1 and MRE, can be activated by Zn-addition but not by Cd.[13] This suggests that MTF-1 can actually act as a Zn sensor that responds directly to Zn, and there should be a mechanism whereby MTF-1 responds to heavy metals other than Zn in mammalian cells. Zhang *et al*. have recently proposed that Zn-MT is a mediator of the Cd-, Cu- and H_2O_2 signal.[14] They found that Cd could promote DNA binding of and transcriptional activation by MTF-1 *in vitro* when

Zn-MT was supplied in the reaction. It is well known that MTF-1 knockout is lethal, and if Zn-MT is critical for the activation of MTF-1, why can MT-knockout mice develop normally? The authors referred to possible participation of other MT subfamilies, but *in vivo* evidence is necessary for elucidating the involvement of MTs in MTF-1 activation.

Domains Responsive to Heavy Metals

It has not been determined which region(s) of the MTF-1 protein is engaged in the heavy metal response. However, the zinc finger domain containing six C_2H_2 type zinc finger motifs is now believed to be the most important for MTF-1 to respond to Zn. Of these six finger motifs, the first, fifth, and sixth fingers are suspected to be involved in the sensory system for Zn.[15–17] The fifth and sixth fingers in particular have lower affinity to Zn compared with other fingers in MTF-1.[15] Functional assays also indicate that the roles of these fingers are distinct from others.[14,16,18] In addition to the finger domain, the participation of other regions of MTF-1 has also been reported, such as the N-terminal domain,[18] the region downstream of Zn fingers,[3] and a well-conserved cysteine cluster structure (CxCxCxC) located in the C-terminal domain.[19] Taken together, it seems likely that the response of MTF-1 to heavy metals is not attributable to the restricted region of MTF-1, but requires the entire protein structure, including intramolecular interactions between various regions.

Unlike heavy metals that activate MTF-1 to induce MT-I/II, Cr^{6+} does not induce MT-I/II; furthermore, the metal down-regulates the MT-induction by Zn and Cd.[20] Majumder *et al*. have shown that such effects of Cr^{6+} are mediated *via* modulation of the MTF-1 function without affecting its DNA binding capacity.[20] They also suggested that Cr^{6+} interferes with the function of three transactivation domains of MTF-1. These results indicate that MTF-1 can be a target of toxic chemicals as well as the activator of genes for cell protection.

Involvement of the Signal Transduction Pathways

Heavy metal signals converging upon MTF-1 may involve signal transduction pathways. LaRochelle *et al*. and Saydam *et al*. have shown that MTF-1 was metabolically phosphorylated and that Cd or Zn-induced transcriptional activation *via* MRE was inhibited by various kinase inhibitors,[21,22] suggesting the involvement of multiple kinase signaling cascades including tyrosine-specific protein kinases, phosphoinositide 3-kinase, protein kinase C, c-Jun N-terminal kinase, and casein kinase. Although it is necessary to demonstrate direct evidence for alterations of MTF-1 function by phosphorylation, it seems likely that the biological actions of heavy metals are mediated in part by the signal transduction pathways. Both groups have also shown that the DNA binding ability of MTF-1 was not affected by the phosphorylation, indicating that transcriptional activation by MTF-1 can be controlled at two steps, DNA binding and the subsequent process.

In conclusion, the modes of transduction of heavy-metal signals to MTF-1 may be more complicated than those considered initially. Since such transduction pathways are thought to reflect the intracellular actions of each heavy metal, elucidation of the mechanism of MTF-1 activation should provide valuable information for research on the biological effects of heavy metals.

TRANSCRIPTIONAL SUPPRESSION OF THE MT-III GENE

In contrast to MT-I/II that is expressed ubiquitously, the expression of MT-III is mainly observed in brain,[23–25] although its existence has been reported in other tissues such as kidney[26] and the male reproductive system.[27] In addition, MT-III is not induced by heavy metals. Although the upstream region of the mouse MT-III gene contains multiple MREs, they are thought to be nonfunctional because MTF-1 cannot bind to them *in vitro*.[28]

Imagawa *et al*. have pointed out the existence of a 25 × CTG repeat in the upstream region of the mouse MT-III gene, and have shown that deletion of the CTG repeat from the promoter region enhanced transcription of a reporter gene fused downstream.[29] Inversely, fusion of the repeat to the promoter (–257–+57) inhibited transcription of the reporter gene, indicating that the repeat functions as a silencer. On the other hand, Watabe *et al*. have found that a sequence similar to the JC virus silencer exists downstream of the CTG repeat, and have shown the sequence contributed to the suppression of MT-III gene expression in primary glial cells.[30] However, Faraonio *et al*. have argued against the silencer function of these sequences because the transcriptional suppression by these sequences is not reproducible.[28] They suggested that regulatory sequences for the MT-III gene are located more than 2453-bp upstream from the transcription start site.

DNA methylation in the promoter region is

known to be the cause of the inability to induce MT-I/II in certain lymphoid cells.[31,32] As to the MT-III gene, Faraonio *et al*. have reported there is no difference in the methylation pattern of the promoter region between permissive and non-permissive cells.[28,33] However, DNA methylation of regions other than upstream of the MT-III gene may participate in transcriptional suppression of the gene. Deng *et al*. have analysed the methylation status of the CpG sites in the human MT-III gene using various gastric carcinoma cells[33] in which the MT-3 gene is markedly downregulated as compared with normal gastric epithelial tissues.[34] They have shown that the extent of DNA methylation of the CpG-island in the first intron of the MT-III gene is correlated with the inducibility of the gene in various gastric carcinomas.[33] Therefore, gene silencing by the higher order structure of chromatin may be involved in the tissue-specific expression of the MT-III gene.

At present, transcriptional regulation of the MT-III/IV genes, especially the MT-IV gene, is poorly understood compared with the MT-I/II genes. It is necessary to check the validity of the suppression mechanisms in various cell systems.

REFERENCES

1) Otsuka, F. and Koizumi, S. (1998) Mechanism of heavy metal-inducible gene transcription. In *Toxicant-Receptor Interactions and Modulation of Gene Expression* (Denison, M. S. and Helferich, W. G., Eds.), Taylor & Francis, Philadelphia, pp. 143–158.
2) Otsuka, F. (2001) Molecular mechanism of the metallothionein gene expression mediated by metal-responsive transcription factor 1. *J. Health Sci.*, **47**, 513–519.
3) Müller, H. P., Brungnera, E., Georgiev, O., Badzong, M., Muller, K. H. and Schaffner, W. (1995) Analysis of the heavy metal-responsive transcription factor MTF-1 from human and mouse. *Somat. Cell Mol. Genet.*, **21**, 289–297.
4) Auf der Maur, A., Belser, T., Elgar, G., Georgiev, O. and Schaffner, W. (1999) Characterization of the transcription factor MTF-1 from the Japanese pufferfish (Fugu rubripes) reveals evolutionary conservation of heavy metal stress response. *Biol. Chem.*, **380**, 175–185.
5) Otsuka, F., Okugaito, I., Ohsawa, M., Iwamatsu, A., Suzuki, K. and Koizumi, S. (2000) Novel responses of ZRF, a variant of human MTF-1, to in vivo treatment with heavy metals. *Biochim. Biophys. Acta*, **1492**, 330–340.
6) Zhang, B., Egli, D., Georgiev, O. and Schaffner, W. (2001) The Drosophila homolog of mammalian zinc finger factor MTF-1 activates transcription in response to heavy metals. *Mol. Cell. Biol.*, **21**, 4505–4514.
7) Fürst, P., Hu, S., Hackett, R. and Hamer, D. (1988) Copper activates metallothionein gene transcription by altering the conformation of a specific DNA binding protein. *Cell*, **55**, 705–717.
8) Heuchel, R., Radtke, F., Georgiev, O., Stark, G., Aguet, M. and Schaffner, W. (1994) The transcription factor MTF-1 is essential for basal and heavy metal-induced metallothionein gene expression. *EMBO J.*, **13**, 2870–2875.
9) Koizumi, S., Yamada, H., Suzuki, K. and Otsuka, F. (1992) Zinc-specific activation of a HeLa cell nuclear protein which interacts with a metal responsive element of the human metallothionein-IIA gene. *Eur. J. Biochem.*, **210**, 555–560.
10) Bittel, D., Dalton, T., Samson, S. L., Gedamu, L. and Andrews, G. K. (1998) The DNA binding activity of metal response element-binding transcription factor-1 is activated in vivo and in vitro by zinc, but not by other transition metals. *J. Biol. Chem.*, **273**, 7127–7133.
11) Koizumi, S., Suzuki, K., Ogra, Y., Yamada, H. and Otsuka, F. (1999) Transcriptional activity and regulatory protein binding of metal-responsive elements of the human metallothionein-IIA gene. *Eur. J. Biochem.*, **259**, 635–642.
12) Palmiter, R. D. (1994) Regulation of metallothionein genes by heavy metals appears to be mediated by a zinc-sensitive inhibitor that interacts with a constitutively active transcription factor, MTF-1. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 1219–1223.
13) Daniels, P. J., Bittel, D., Smirnova, I. V., Winge, D. R. and Andrews, G. K. (2002) Mammalian metal response element-binding transcription factor-1 functions as a zinc sensor in yeast, but not as a sensor of cadmium or oxidative stress. *Nucleic. Acids Res.*, **30**, 3130–3140.
14) Zhang, B., Georgiev, O., Hagmann, M., Gunes, C., Cramer, M., Faller, P., Vasak, M. and Schaffner, W. (2003) 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.
15) Chen, X., Chu, M. and Giedroc, D. P. (1999) 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.

16) Bittel, D. C., Smirnova, I. V. and Andrews, G. K. (2000) Functional heterogeneity in the zinc fingers of metalloregulatory protein metal response element-binding transcription factor-1. *J. Biol. Chem.*, **275**, 37194–37201.
17) Jiang, H., Daniels, P. J. and Andrews, G. K. (2003) Putative zinc-sensing zinc fingers of metal-response element-binding transcription factor-1 stabilize a metal-dependent chromatin complex on the endogenous metallothionein-I promoter. *J. Biol. Chem.*, **278**, 30394–30402.
18) Koizumi, S., Suzuki, K., Ogra, Y., Gong, P. and Otuska, F. (2000) Roles of zinc fingers and other regions of the transcription factor human MTF-1 in zinc-regulated DNA binding. *J. Cell. Physiol.*, **185**, 464–472.
19) Chen, X., Zhang, B., Harmon, P. M., Schaffner, W., Peterson, D. O. and Giedroc, D. P. (2004) A novel cysteine cluster in human metal-responsive transcription factor 1 is required for heavy metal-induced transcriptional activation in vivo. *J. Biol. Chem.*, **279**, 4515–4522.
20) Majumder, S., Ghoshal, K., Summers, D. M., Bai, S., Datta, J. and Jacob, S. T. (2003) Chromium(VI) downregulates heavy metal-induced metallothionein gene transcription by modifying transactivation potential of the key transcription factor MTF-1. *J. Biol. Chem.*, **278**, 26216–26226.
21) LaRochelle, O., Gagne, V., Charron, J., Soh, J. W. and Seguin, C. (2001) Phosphorylation is involved in the activation of metal-regulatory transcription factor 1 in response to metal ions. *J. Biol. Chem.*, **276**, 41879–41888.
22) Saydam, N., Adams, T. K., Steiner, F., Schaffner, W. and Freedman, J. H. (2002) Regulation of metallothionein transcription by the metal-responsive transcription factor MTF-1: identification of signal transduction cascades that control metal-inducible transcription. *J. Biol. Chem.*, **277**, 20438–20445.
23) Uchida, Y., Takio, K., Titani, K., Ihara, Y. and Tomonaga, M. (1991) The growth inhibitory factor that is deficient in the Alzheimer's disease brain is a 68 amino acid metallothionein-like protein. *Neuron*, **7**, 337–347.
24) Palmiter, R. D., Findley, S. D., Whitmore, T. E. and Durnam, D. M. (1992) MT-III, a brain-specific member of the metallothionein gene family. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 6333–6337.
25) Palmiter, R. D. (1998) The elusive function of metallothioneins. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 8428–8430.
26) Hoey, J. G., Garrett, S. H., Sens, M. A., Todd, J. H. and Sens, D. A. (1997) Expression of MT-3 mRNA in human kidney, proximal tubule cell cultures, and renal cell carcinoma. *Toxicol. Lett.*, **92**, 149–160.
27) Moffatt, P. and Seguin, C. (1998) Expression of the gene encoding metallothionein-3 in organs of the reproductive system. *DNA Cell Biol.*, **17**, 501–510.
28) Faraonio, R., Moffatt, P., Larochelle, O., Schipper, H. M. S-Arraud, R. and Seguin, C. (2000) Characterization of cis-acting elements in the promoter of the mouse metallothionein-3 gene. Activation of gene expression during neuronal differentiation of P19 embryonal carcinoma cells. *Eur. J. Biochem.*, **267**, 1743–1753.
29) Imagawa, M., Ishikawa, Y., Shimano, H., Osada, S. and Nishihara, T. (1995) CTG triplet repeat in mouse growth inhibitory factor/metallothionein III gene promoter represses the transcriptional activity of the heterologous promoters. *J. Biol. Chem.*, **270**, 20898–20900.
30) Watabe, M., Gross, S., Lawyer, C., Brewer, G. J., Mashimo, T. and Watabe, K. (1997) Sequence and functional analysis of the 5′-flanking region of the mouse growth inhibitory factor gene. *Cell. Mol. Neurobiol.*, **17**, 235–243.
31) Compere, S. J. and Palmiter, R. D. (1981) DNA methylation controls the inducibility of the mouse metallothionein-I gene lymphoid cells. *Cell*, **25**, 233–240.
32) Majumder, S., Ghoshal, K., Li, Z., Bo, Y. and Jacob, S. T. (1999) Silencing of metallothionein-I gene in mouse lymphosarcoma cells by methylation. *Oncogene*, **18**, 6287–6295.
33) Deng, D., El-Rifai, W., Ji, J., Zhu, B., Trampont, P., Li, J., Smith, M. F. and Powel, S. M. (2003) Hypermethylation of metallothionein-3 CpG island in gastric carcinoma. *Carcinogenesis*, **24**, 25–29.
34) El-Rifai, W., Frierson, H. F., Jr., Harper, J. C., Powel, S. M. and Knuutila, S. (2001) Expression profiling of gastric adenocarcinoma using cDNA array. *Int. J. Cancer*, **92**, 832–838.