Putting its fingers on stressful situations: the heavy metal-regulatory transcription factor MTF-1

P. Lichtlen and W. Schaffner***

Summary

It has been suggested that metallothioneins, discovered about 45 years ago, play a central role in heavy metal metabolism and detoxification, and in the management of various forms of stress. The metal-regulatory transcription factor-1 (MTF-1) was shown to be essential for basal and heavy metal-induced transcription of the stress-responsive *metallothionein-I* and *metallothionein-II*. Recently it has become obvious that MTF-1 has further roles in the transcriptional regulation of genes induced by various stressors and might even contribute to some aspects of malignant cell growth. Furthermore, *MTF-1* is an essential gene, as mice null-mutant for *MTF-1* die in utero due to liver degeneration. We describe here the state of knowledge on the complex activation of MTF-1, and propose a model with MTF-1 as an interconnected cellular stress-sensor protein involved in heavy metal metabolism, hepatocyte differentiation and detoxification of toxic agents. *BioEssays* 23:1010–1017, 2001.

Introduction

Trace amounts of several heavy metals are essential for life. Among these, the transition heavy metal zinc is most abundant in most living organisms.[1] A great number of proteins, including zinc-containing enzymes and transcription factors that contain so-called zinc finger motifs, as well as other regulatory nuclear proteins with ring finger domains or LIM domains, contain single or multiple zinc ions as integral components. A deficiency in zinc can lead to severe growth retardation, immune deficiency, impaired hair growth, and fertility problems due to reduced sperm production.[2–4] A genetic defect in zinc uptake results in acrodermatitis enteropathica.[5,6] About 45 years ago, the group of Bert Vallee discovered metallothioneins, a group of small, cysteine-rich proteins that are particularly abundant in the kidney and the liver. They have the ability to bind heavy metals such as zinc, cadmium, copper, nickel and cobalt (reviewed in Ref. 7). Metallothioneins are involved in homeostatic regulation of zinc concentrations and also for the detoxification of non-essential heavy metals. As an example of the latter, mammals store all cadmium taken up in food in a form tightly bound to metallothioneins, where it remains with a half-life of approximately 15 years in humans.[8] The expression of the major metallothionein genes (*MT-I* and *MT-II*) is induced at the level of transcription by heavy metal load.[9] As shown by Palmiter and colleagues, the promoter region of these metallothionein genes contain so-called metal responsive elements (MREs) that can confer metal-inducibility to any reporter gene when placed in a promoter position[10,11] or at a remote enhancer position.[12] Several laboratories, including ours, therefore began to search for an MRE-binding factor(s). In 1988, MTF-1 (MRE-binding transcription factor, or metal-regulatory transcription factor) was identified as a candidate protein that indeed required elevated zinc concentrations for optimal DNA binding.[13] Subsequently, *MTF-1* was cloned and characterized.[14] MTF-1 is a ubiquitously expressed zinc finger factor essential for basal and heavy metal-induced expression of metallothioneins (Figure 1).[15] The basal expression of *metallothionein-I* and *metallothionein-II* is reduced about hundred-fold in ES cells, whereas it is reduced less, but still significantly in other cell lines lacking MTF-1. *MTF-1* expression is particularly high in the testes,[16] although it is not known whether this serves a specific function, or whether it reflects a general tendency for transcriptional regulatory proteins to be highly expressed in testicular tissue.[17]

Mouse MTF-1 is a 675 amino acid protein with a calculated molecular mass of 72.5 kDa.[14] Its N-terminal part is followed by six zinc fingers of the Cys_2-His_2 type that harbor the DNA-binding activity and sense the level of zinc.[15] MTF-1, unlike other zinc-finger transcription factors, is reversibly activated to bind DNA in response to changes in zinc status and the interaction of the protein with zinc ions occurs with the zinc-finger domain.[18] Three groups have independently shown that the six zinc fingers are functionally different.[19–21] Studies with the isolated MTF-1 zinc-finger domain suggest that the four N-terminal zinc fingers are required for high affinity and specific binding to the seven-base-pair core consensus MRE

Institute of Molecular Biology, University of Zurich
Funding agency: The Schweizerische Nationalfonds and by the Kanton Zürich.
*Correspondence to: W. Schaffner, Institute of Molecular Biology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.
**Current address: ESBATech AG, Winterthurerstrasse 190, CH-8057 Zürich

motif (TGCRCNC). This protein–DNA interaction is subject to allosteric structural modulation by the C-terminal zinc fingers 5 and 6, which have a lower affinity for zinc. The latter zinc fingers bind at or near the gGCCc sequence (small letters stand for poorly conserved bases) of the consensus MRE motif (ctNTGCRCNCgGCCc), and apparently induce a local conformational change in the DNA from a typical B-form structure to an A-form conformation.[19,22] Partly in contrast, data by Bittel et al. suggest that, in the context of the entire mouse MTF-1 protein, zinc-fingers 2–4 constitute the core DNA-binding domain and fingers 5 and 6 are unnecessary for DNA binding in vitro and metal-dependent function in transiently transfected cells.[21] The same group also found that deletion of zinc-finger 1 results in a protein that binds DNA constitutively in vitro and conclude that zinc-finger 1 is a unique zinc-sensing domain.[21] Taken together, the model proposed by Andrews and colleagues suggests that finger 1, unless it has bound zinc, prevents adjacent fingers from binding to DNA. Therefore finger 1 may be active under normal conditions, whereas fingers 5 and 6 may serve to sense high zinc concentrations. The question of whether zinc finger 1 and/or zinc fingers 5 and 6 are important for sensing the zinc concentrations under physiological conditions certainly requires further investigation (for a detailed review on the biochemical properties of MTF-1 see Giedroc et al.[23]). At the C terminus, MTF-1 contains three distinct activation domains: an acidic, a proline-rich, and a serine/threonine-rich domain, each with distinct properties in transcriptional activation.[14,24]

Not unexpectedly, MTF-1 is conserved in evolution. The human and mouse homologues are highly similar with a 93% amino acid identity.[25] However, the mouse protein is 78 amino acids shorter in the C-terminal region, relative to MTF-1 from human and apparently from most other mammals.[25] In MTF-1 of the pufferfish *Fugu rubripes*, the zinc fingers and two further domains, one upstream and one downstream of the zinc fingers,[26] are well conserved, while the three activation domains are only partly conserved. In *Drosophila*, which harbors the most distant MTF-1 homologue examined so far, the six zinc fingers are again conserved, but otherwise similarity is confined to several short patches throughout the rest of the protein.[27] From humans to *Drosophila*, activation of transcription is mediated via metal-responsive promoter elements (MREs), the short DNA-recognition sequences of MTF-1. In addition, MTF-1 probably binds to the cognate DNA sequence as a monomer, since the MRE does not contain any sequence symmetry; in addition, the binding of the zinc-finger domain to the MRE occurs in a specific orientation (see above).[19]

Activation of MTF-1

When cells are treated with heavy metals, MTF-1 is activated, binds to MREs, and induces transcription.[14,15] What is the mechanism responsible for this activation? Northern blots reveal a strong increase of metallothionein mRNAs, while transcripts of MTF-1 itself are only marginally, if at all, elevated under heavy metal load, suggesting a post-transcriptional activation of the MTF-1 regulatory protein.[16] In accordance with this, the promoter of the mouse *MTF-1* gene contains no metal-responsive elements.[16] Rather, the induction is mediated at the post-translational level, including transport of MTF-1 to the nucleus (see below), and to a smaller extent at the level of translation/protein stability. The finding that MTF-1 requires an elevated concentration of zinc for strong binding to DNA immediately suggested a mechanism of MTF-1 activation, namely allosteric regulation of DNA binding via binding of metals to the transcription factor itself (see above).[13–15] Indeed, such a mode of MTF-1 activation was recently suggested to play an important role for metallothionein gene activation in vivo.[28] MTF-1 and zinc was found to be required for the heightened expression of *metallothionein-I* in the visceral endoderm of the developing mouse embryo. This suggests that zinc modulates the ability of MTF-1 to transactivate gene expression by acting as a signaling ligand to induce cell-specific metallothionein expression in visceral endoderm cells. Furthermore, the study showed that MTF-1, at least in this cell type, cooperates with the transcription factor USF1 to regulate *metallothionein-I*, which implies that MTF-1 might need additional cofactors or coactivators to execute its full transcriptional activity.

In marked contrast to the strong activation by zinc in cell-free extracts, treatment by other heavy metals (cadmium, copper, nickel, lead) abolishes MTF-1 function, however, none of these metals can substitute for zinc in DNA binding, even though they readily induce metallothionein gene transcription in cultured cells.[15,18,29] This paradox can be explained in two ways: either, these other heavy metals have their own pathway of transcriptional induction, possibly via regulatory proteins other than MTF-1, or the induction is an indirect one and still somehow involves MTF-1. Palmiter speculated that treatment by heavy metals other than zinc would release zinc from an intracellular store, which would induce transcription. In other words, cadmium and other heavy metals would ultimately all act via zinc release.[30,31] Andrews and colleagues also found that, in a human neuroblastoma cell line that hardly expresses MTF-1, cadmium is able to activate *MT* genes, suggesting a mechanism distinct from that of MTF-1/zinc activation.[32] Due to the high concentration of zinc within the living cell, most of the metallothionein is normally saturated by zinc, even though it has a much higher affinity for scarce heavy metals such as cadmium and copper. Therefore, the most-likely scenario is a substitution of zinc by cadmium in cellular zinc-storage proteins such as metallothioneins, and/or a displacement of zinc from extracellular sites, e.g. albumin, leading to a concomitant activation of MTF-1 by the released zinc. The response of MTF-1 is not confined to heavy metals, however. Dalton et al.[33] have found that in hepatoma cells, MTF-1 is

also activated by treatment of the cells with hydrogen peroxide. In agreement, we found that cells lacking MTF-1 are more sensitive to hydrogen peroxide than their wild-type counterparts.[34] Of particular interest in this context is a recent report that *MTF-1* was greatly overexpressed in a radio-resistant cervical carcinoma cell line relative to the expression in a radiosensitive cell line of the same origin.[35] These findings imply a role for MTF-1 in the regulation of genes involved in the cellular response to oxidative stress.

Andrews and colleagues recently discovered another level of complexity in the activation pathway of MTF-1 in that MTF-1 is mostly cytoplasmic, but is translocated to the nucleus upon metal induction.[36] We have analyzed this phenomenon further, and found that nuclear transport is induced by a variety of conditions. Besides growth factors, a number of stress conditions induce the transfer of the protein to the nucleus, namely, heavy metal load by zinc or cadmium, heat shock, hydrogen peroxide, low extracellular pH, and inhibition of protein synthesis by cycloheximide.[37] MTF-1 is also translocated to the nucleus in response to receptor-mediated heme transport from hemopexin, which probably induces surface redox processes and Cu(I)-dependent signalling.[38] Curiously, only heavy metal load, but none of the other conditions, results in transcriptional activation of a *metallothionein-I* promoter in HeLa cells. This raises the question of whether nuclear import of MTF-1 under stress conditions other than heavy metal load is at all physiologically relevant, or merely represents a side effect of another stress response. Nevertheless, it is an attractive possibility that MTF-1, in stress situations other than metal load, assists in the transcription of genes that are primarily activated by other transcription factors. Another question concerns the role of protein phosphorylation in the activation of MTF-1. Staurosporine, a broad-range PKC inhibitor, interferes with heavy metal-responsive transcription,[39] and experiments to extend these findings are in progress in our laboratory.

MTF-1 knockout

There are four types of metallothionein genes in the mouse. *MT-I* and *MT-II* are ubiquitously expressed and stress-inducible, while expression of *MT-III* and *MT-IV* is confined to specific cell types. Because of the high level of expression of the *metallothionein-I* and *metallothionein-II* upon metal stress, oxidative stress and a variety of other conditions, it seemed possible that a simultaneous knockout of *metallothionein-I* and *metallothionein-II*, which are separated in the genome by a mere six kilobases, would result in a severe phenotype. Somewhat unexpectedly, mice lacking *MT-I* and *MT-II* grow normally under laboratory conditions.[40,41] However, when dams are reared on severely zinc-deficient diet, a lack of MT-I/MT-II leads to increased embryonic lethality and morphological abnormalities.[42] In a separate study, the brain-specific *MT-III* was eliminated, again without causing a phenotype other than a higher susceptibility to induced seizures.[43] We have undertaken the targeted disruption of the major transcriptional activator of metallothionein genes, MTF-1, and found this knockout to be lethal. A closer investigation revealed that *MTF-1* knockout embryos die in utero around embryonic day (E) 13-14, with degeneration of the embryonic hepatocytes as a hallmark.

In addition, cultured knockout cells taken from embryos before the onset of the lethal crisis show a higher susceptibility to the effects of cadmium and hydrogen peroxide.[34] In this study, we further noted a similarity of the knockout phenotype to that of *c-jun*, *p65/RelA* and *HGF/SF/c-met*.[44–48] In the meantime, knockout embryos for the *SAPK/ERK kinase-1 (SEK-1)* also yielded a very similar phenotype of liver degeneration at about this stage.[49] This has raised the question of whether the embryonic liver at this stage might be particularly susceptible to a lack of stress-involved regulators. SEK-1/MKK4 is a MAP kinase kinase that has been shown to participate in vitro in two stress-activated cascades that terminate with the p38 and SAPK/JNK kinases, which in turn lead to phosphorylation and activation of transcription factors such as c-jun.[50,51] Accordingly, *SEK-1* null mutant mouse embryos die as a result of abnormal liver formation and hemorrhage between E12.5 and 14.5.[49] The striking similarity of this phenotype to that of *c-jun*-deficient embryos suggests a model in which the stress-activated MAPK cascades lead to *c-jun* activation, which is essential for normal liver development in vivo.[44] These phenotypes are very similar, though not identical to the one of *MTF-1* knockout mice as previously mentioned.[34] Although MTF-1 is probably not activated by the same kinases as c-jun, it is obvious that it is phosphorylated in vivo (Nurten Saydam and W.S. unpublished data), most likely upon activation of an isoform of PKC. Taken together, activation of MTF-1 under non-metal-induced conditions, might work in a similar way to the activation of c-jun, potentially even leading to transcriptional activation of common target genes. Nevertheless, the fact that MTF-1 levels in *c-jun* knockout fibroblasts are not changed (and vice versa Ref. 34) speaks in favour of a parallel, rather than an epistatic relationship between these two essential transcription factors. In addition, it should also be pointed out that the phenotypes of the other gene knockouts mentioned above, although generally similar, nevertheless show marked differences to the one of *MTF-1* knockout mice. For example, the *p65/RelA* knockout phenotype manifests itself via apoptotic cell death, while that of *MTF-1* knockouts results primarily in necrotic cell death of hepatocytes. In addition, liver degeneration of the *p65/RelA* knockout can be rescued by concommitant knockout of *TNF* or *TNF-receptor* genes, suggesting that apoptosis is brought about by a loss of a p65/RelA-mediated rescue circuit against apoptotic signaling.[52]

The lethal embryonic phenotype of *MTF-1* knockout mice prevents investigation of the role of MTF-1 in the development

of tissues other than the liver later than E14. Neural cells null-mutant for *MTF-1* grafted into the brains of wild-type mice survive for several weeks[53] and MTF-1 knockout fibroblasts survive in cell culture.[34] Definitive answers to the role of MTF-1 for development and function of other tissues are expected from so-called conditional knockout studies that are now under way, whereby a *MTF-1* cDNA with rescue ability (see below) is eliminated by site-specific recombination via flanking loxP sites and tissue-specific inducible expression of cre-recombinase. These studies based on the generation of transgenic, MTF-1-expressing mice on a mouse background knockout for endogenous MTF-1, in which the lethal phenotype can be fully rescued by expression of the transgene under the control of the generally-active, human ubiquitin promoter. Indeed, preliminary data using such conditional knockout animals strongly suggest, that MTF-1 is not essential for hepatocyte function in adult mice under non-stress conditions. This argues for a development-specific role of MTF-1 in these cells (P.L., Ying Wang, W.S., unpublished results).

So far, the only evidence for an MTF-1 requirement under non-stress condition is the liver degeneration phenotype. It might well be, however, that further phenotypes would emerge at stages later than E14 in the brain, or in the pancreas, which has the highest expression of metallothionein genes of all organs and has been shown to require the regulated expression of metallothionein genes,[54–56] or in testes, which highly express *MTF-1*.[16]

MTF-1 target genes

The observation that the double knockout of *MT-I* and *MT-II* was viable while the *MTF-1* knockout was lethal, suggested that MTF-1 activates important genes other than those encoding metallothioneins. *MTF-1* knockout cells show an increased sensitivity towards H_2O_2.[34] Glutathione (GSH) can substitute, in a less specific way, for metallothioneins in the cellular defense against heavy metals, toxic compounds and reactive oxygen intermediates.[57,58] The finding that the gene for gamma glutamyl-cysteine synthetase heavy chain (γGCS_{hc}), a key enzyme in glutathione synthesis, also contains metal-responsive elements, was therefore of particular interest. Indeed, transcript levels of γGCS_{hc} were found to be reduced in E13.5 MTF-1 null mutant embryos.[34] Nevertheless, we recently found GSH levels in E12.5 embryonic MTF-1 knockout livers to be at least as high as in wild-type littermates.[59] From this result, we conclude that the *MTF-1* knockout phenotype cannot be explained by any decrease in γGCS_{hc} expression, although we cannot formally exclude additional unknown functions of this enzyme in addition to synthesis of GSH. In the same study, we presented *C/EBP*α and α*-fetoprotein (AFP)* as likely in vivo target genes of MTF-1. Both genes provide new clues to the molecular pathogenesis of the knockout phenotype: *C/EBP*α is involved in the maintenance of the differentiated, non-proliferating state of hepatocytes, apparently by inducing the antiproliferative protein p21.[60] In addition, it plays a role in cellular energy metabolism and, most interestingly, it is involved in the cellular stress response, as indicated by its induction during the so-called acute phase response.[61,62] AFP, a member of the albuminoid protein superfamily predominantly expressed in mouse embryos, plays various, interesting biological roles.[63] First of all, it is responsible for maintenance of the colloid-osmotic pressure, and as such its downregulation in *MTF-1* knockout embryos could explain the late stages of the knockout phenotype, which are characterized by generalized edema.[34] In addition, AFP acts as a scavenger for heavy metals and reactive oxygen intermediates (ROI), and also influences developmental processes.[63] *Zinc transporter-1 (ZnT-1)* and *tear lipocalin* have been described recently as two further target genes of MTF-1.[59,64] While tear lipocalin does not appear to be involved in the manifestation of the *MTF-1* knockout phenotype, ZnT-1 might well contribute to it, as suggested by ZnT-1 function in zinc metabolism and its expression in the liver. Furthermore, *ZnT-1* was shown to be an in vivo target gene of MTF-1, notably in the midgestational visceral yolk sac and in the placenta.[64] Taken together, MTF-1 is a crucial transcriptional regulator for basal expression of at least three genes (*MT-I, MT-II, ZnT-1*) involved in zinc metabolism during mouse development (Figure 2).[34,64] In this context, it will be interesting to see if the deleterious effects of maternal zinc deficiency, which have been observed in *MT-I/-II* knockout mice,[42] will be accentuated in *MTF-1* knockout mice.

To date, the role for metallothioneins in cancer remains controversial. Nevertheless, it is obvious that at least in some forms of malignant human tumors, a high expression of metallothionein genes is correlated with resistance to therapy and a poor prognosis.[65,66] Such tumors usually grow under hypoxic conditions and induce angiogenesis. In line with such a scenario, the groups of Murphy and Andrews have recently found another function for MTF-1, namely the induction of human *metallothionein-IIA* and mouse *metallothionein-I* in response to hypoxia.[67] Of particular interest in this context are the findings of the Murphy group, in a collaboration with our laboratory, that under hypoxic conditions expression of placenta growth factor (PlGF), encoding a member of the VEGF-family of angiogenetic factors, is induced in an MTF-1-dependent manner.[68] Furthermore, preliminary results of xenograft studies suggest that *MTF-1* deficiency retards the formation of tumors by ras-transformed fibroblasts, resulting in significantly reduced tumor masses when compared to MTF-1 expressing tumors at two weeks after injection of the cells into nude mice (Brian Murphy, P.L., W.S., unpublished data). Taken together, our findings suggest that the *PlGF* gene is responsive to hypoxia, and that this activation is mediated by MTF-1. This implies a contribution of MTF-1 to some aspects of malignant cellular behaviour, which is underlined by an

independent study on its overexpression in a radiation-resistant tumor cell line[35] (see above).

Conclusions

MTF-1 is a transcriptional activator that plays an important role in the regulation of genes involved in heavy metal homeostasis. It functions through activation via heavy metal load and zinc binding and is apparently also activated by oxidative stress. Nuclear translocation as well as phosphorylation of MTF-1 increase the complexity of its functional regulation. Most interestingly, *MTF-1* null-mutant mice die in utero due to degeneration of the embryonic hepatocytes.

Several questions about the role of MTF-1 remain unanswered to date. First of all, the mechanism(s) underlying the lethal liver degeneration of mouse embryos remains elusive. Is it, as we speculated earlier on,[34] that the embryonic liver is decaying from intoxication because metallothionein genes and other stress-involved genes are not sufficiently expressed? However, latest results rather suggest that, MTF-1 may be essential for the control of a specific stage of liver development, and thereafter would be required only to handle particular stress situations (see above). Interestingly, it has been speculated that the evolutionary driving force for proteins known as stress-response regulators is actually their role in development, as exemplified by the tumor suppressor and stress factor p53.[69]

The role of the cytoplasmic-nuclear shuttling of MTF-1 and the requirement for phosphorylation (and dephosphorylation) also remain to be analyzed in more detail. Many, but not all, of these questions can be readily studied in *Drosophila*, where a homologue of MTF-1 has recently been found.[27] In conclusion, it appears that MTF-1 is an essential zinc finger factor

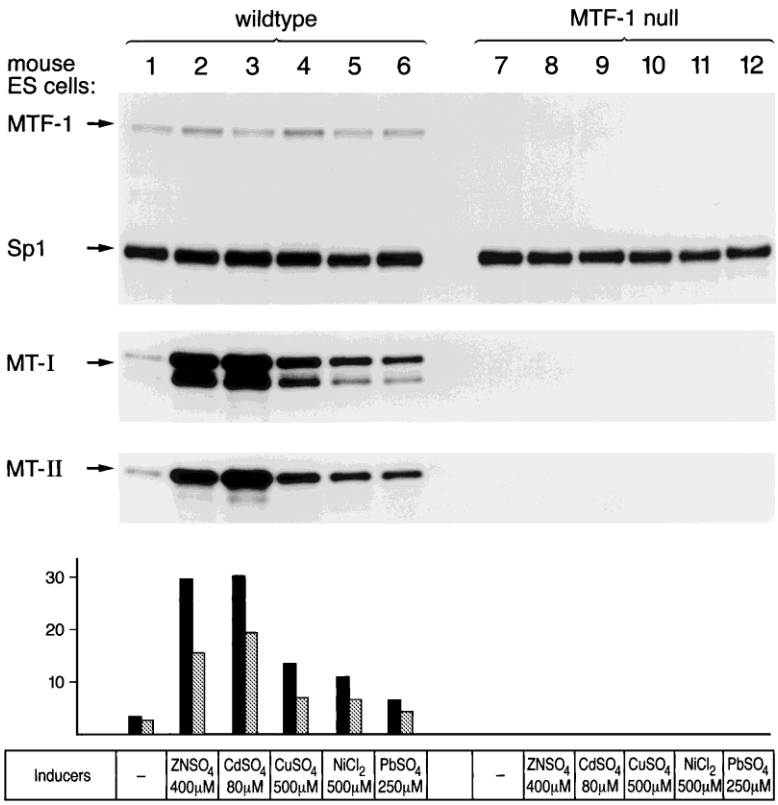
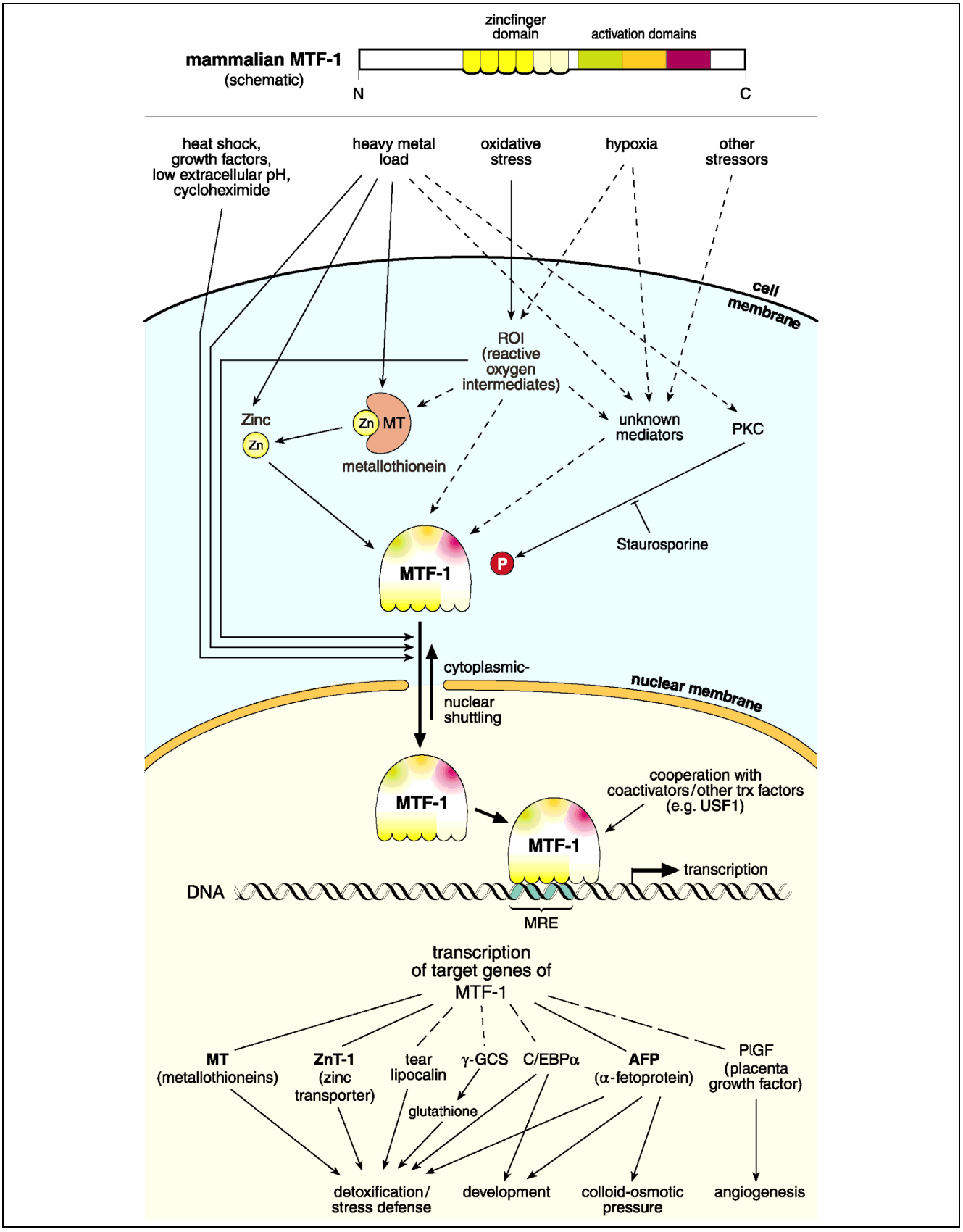


Figure 1. Loss of metallothionein gene regulation in *MTF-1*$^{-/-}$ embryonic stem cells. Transcript levels of MTF-1, metallothionein I and metallothionein II were determined in *MTF-1*$^{+/+}$ and *MTF-1*$^{-/-}$ ES cells. Each lane represents protected RNA segments obtained after simultaneously hybridizing 20 μg of cytoplasmic RNA with MTF-1, Sp1, metallothionein I and metallothionein II antisense RNA probes. Sp1 transcript levels were used as internal controls, because they are not affected by heavy metal treatment. Before harvesting, cells were treated with different metal salts, as indicated. In *MTF-1*$^{+/+}$ ES cells, a several-fold induction of both *metallothionein I* and *metallothionein II* can be observed after challenge with different heavy metals (lane 1–6). The transcript levels of Sp1 and MTF-1 itself are at most marginally influenced by this treatment. However, neither basal nor heavy metal-induced transcripts of metallothionein genes can be detected in *MTF-1*$^{-/-}$ ES cells, which lack MTF-1 (lanes 1–7). The residual metallothionein I and II levels in ES cells were determined by PhosphorImager analysis to be less than 1% of the basal level observed in *MTF-1*$^{+/+}$ ES cells (lane 1 versus lanes 7–12). Reproduced with permission from EMBO Journal.[15]


mammalian MTF-1
(schematic)
zincfinger domain
activation domains
N
C
heat shock, growth factors, low extracellular pH, cycloheximide
heavy metal load
oxidative stress
hypoxia
other stressors
cell membrane
ROI (reactive oxygen intermediates)
Zinc
Zn
Zn MT
metallothionein
unknown mediators
PKC
Staurosporine
P
MTF-1
cytoplasmic-nuclear shuttling
nuclear membrane
MTF-1
cooperation with coactivators/other trx factors (e.g. USF1)
MTF-1
transcription
DNA
MRE
transcription of target genes of MTF-1
MT (metallothioneins)
ZnT-1 (zinc transporter)
tear lipocalin
γ-GCS
C/EBPα
AFP (α-fetoprotein)
PlGF (placenta growth factor)
glutathione
detoxification/ stress defense
development
colloid-osmotic pressure
angiogenesis

involved in the response to metal load and apparently some other types of stress. As it is typical for higher eukaryotes, different forms of stress response can produce overlapping patterns of gene activity. Thus, we would not be surprised to find new roles for MTF-1 in a variety of stress conditions other than those evoked by heavy metal, hypoxia, xenobiotic components, and reactive oxygen intermediates. The downside of this ability of cells to cope with various forms of stress, however, may be that the malignant growth and therapy resistance of tumors could potentially be supported by highly active MTF-1 that causes elevated expression of metallothioneins and other cytoprotective proteins.

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References

1. Vallee BL. Zinc: biochemistry, physiology, toxicology and clinical pathology. Biofactors 1988;1:31–36.
2. MacDonald RS. The role of zinc in growth and cell proliferation. J Nutr 2000;130(5S Suppl.):1500S–1508S.
3. Prasad AS. Zinc and immunity. Mol Cell Biochem 1998;188:63–69.
4. Hamdi SA, Nassif OI, Ardawi MS. Effect of marginal or severe dietary zinc deficiency on testicular development and functions of the rat. Arch Androl 1997;38:243–253.
5. Prasad AS. Zinc: an overview. Nutrition 1995;11(1 Suppl.):93–99.
6. Muga SJ, Grider A. Partial characterization of a human zinc-deficiency syndrome by differential display. Biol Trace Elem Res 1999;68:1–12.
7. Kägi JH. Evolution, structure and chemical activity of class I metallothioneins: an overview. In: Suzuki KT, Imura N, Kimura M, eds; Metallothionein III. Basel/Switzerland: Birkhäuser Verlag. 1993.
8. Nordberg M, Nordberg GF. On the role of metallothionein in cadmium induced renal toxicity. EXS 1987;52:669–675.
9. Durnam DM, Palmiter RD. Transcriptional regulation of the mouse metallothionein-I gene by heavy metals. J Biol Chem 1981;256:5712–5716.
10. Stuart GW, Searle PF, Chen HY, Brinster RL, Palmiter RD. A 12-base-pair DNA motif that is repeated several times in metallothionein gene promoters confers metal regulation to a heterologous gene. Proc Natl Acad Sci USA 1984;81:7318–7322.
11. Stuart GW, Searle PF, Palmiter RD. Identification of multiple metal regulatory elements in mouse metallothionein-I promoter by assaying synthetic sequences. Nature 1985;317:828–831.
12. Serfling E, Lubbe A, Dorsch-Hasler K, Schaffner W. Metal-dependent SV40 viruses containing inducible enhancers from the upstream region of metallothionein genes. EMBO J 1985;4:3851–3859.
13. Westin G, Schaffner W. A zinc-responsive factor interacts with a metal-regulated enhancer element (MRE) of the mouse metallothionein-I gene. EMBO J 1988;7:3763–3770.
14. Radtke F, Heuchel R, Georgiev O, Hergersberg M, Gariglio M, Dembic Z, Schaffner W. Cloned transcription factor MTF-1 activates the mouse metallothionein-I promoter. EMBO J 1993;12:1355–1362.
15. Heuchel R, Radtke F, Georgiev O, Stark G, Aguet M, Schaffner W. The transcription factor MTF-1 is essential for basal and heavy metal-induced metallothionein gene expression. EMBO J 1994;13:2870–2875.
16. Auf der Maur A, Belser T, Wang Y, Günes C, Lichtlen P, Georgiev O, Schaffner W. Characterization of the mouse gene for the heavy metal-responsive transcription factor MTF-1. Cell Stress & Chaperones 2000;5:196–206.
17. Schmidt EE, Schibler U. High accumulation of components of the RNA polymerase II transcription machinery in rodent spermatids. Development 1995;121:2373–2383.
18. Dalton TP, Bittel D, Andrews GK. Reversible activation of mouse metal response element-binding transcription factor 1 DNA binding involves zinc interaction with the zinc finger domains. Mol Cell Biol 1997;17:2781–2789.
19. Chen X, Chu M, 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. Biochem 1999;38:12915–12925.
20. Koizumi S, Suzuki K, Ogra Y, Gong P, Otsuka F. Roles of zinc fingers and other regions of the transcription factor human MTF-1 in zinc-regulated DNA binding. J Cell Physiol 2000;185:464–472.
21. Bittel DC, Smirnova IV, Andrews GK. Functional heterogeneity of the zinc fingers of metalloregulatory protein metal response element-binding transcription factor-1. J Biol Chem 2000;275:37194–37201.
22. Chen X, Agarwal A, Giedroc DP. Structural and functional heterogeneity among the zinc fingers of human MRE-binding transcription factor-1. Biochem 1998;37:11152–11161.
23. Giedroc, D.P., Chen, X. and Apuy, J. Metal response element (MRE)-binding transcription factor-1 (MTF-1): Structure, function and regulation. Antiox. Redox Signal., in press.
24. Radtke F, Georgiev O, Müller H-P, Brugnera E, Schaffner W. Functionaldomains of the heavy metal-responsive transcription factor MTF-1. Nucleic Acids Res 1995;23:2277–2286.
25. Brugnera E, Georgiev O, Radtke F, Heuchel R, Baker E, Sutherland GR, Schaffner W. Cloning, chromosomal mapping and characterisation of the human metal-regulatory transcription factor MTF-1. Nucleic Acids Res 1994;22:3167–3173.
26. Auf der Maur A, Belser T, Elgar G, Georgiev O, Schaffner W. Characterization of the transcription factor MTF-1 from the Japanese pufferfish (Fugu rubripes) reveals evolutionary conservation of heavy metal stress response. Biol Chem 1999;380:175–185.
27. Zhang B, Egli D, Georgiev O, Schaffner W. The *Drosophila* homologue of mammalian zincfinger factor MTF-1 activates transcription in response to copper and cadmium. Mol Cell Biol 2001;21:4504–4514.

Figure 2. Overview of known and putative functions of the metal transcription factor MTF-1. Upper part: MTF-1 can be activated by several conditions, notably heavy metals such as zinc, cadmium, copper, nickel, lead (see Fig. 1). Activation can be direct, via zinc, or indirect, via liberation of zinc from metallothionein. Activation is inhibited by Staurosporine, a broad-range inhibitor of protein kinases c (PKCs). Activated MTF-1 is translocated from the cytoplasm to the nucleus in response to heavy metal load and several other conditions, including oxidative stress, heat shock, growth factors, low pH, and cycloheximide. Nuclear translocation and/or transcription activation is accompanied by phosphorylation. Lower part: Within the nucleus, MTF-1 binds specifically to so-called metal responsive elements (MREs), DNA sequence motifs of the consensus TGCRCNC. Target genes with MRE motifs in the upstream/promoter region include *metallothioneins, zinc transporter (ZnT-1), transcription factor C/EBPα, α-fetoprotein, placenta growth factor, γ-GCS$_{hc}$, tear lipocalin/von Ebners gland protein*. Activation of these target genes may be direct, with or without cooperation from other transcription factors such as USF1, or indirect, via transcriptional activation of transcription factors such us C/EBPα, which in turn activate other genes. Uncertain interactions/effects are indicated with dashed lines.

28. Andrews GK, Lee DK, Ravindra R, Lichtlen P, Sirito M, Sawadogo M, Schaffner W. The transcription factors MTF-1 and USF1 cooperate to regulate mouse metallothionein-I expression in response to the essential metal zinc in visceral endoderm cells during early development. EMBO J 2001;20:1114–1122.
29. Saint-Jacques E, April MJ, Seguin C. Structure and metal-regulated expression of the gene encoding *Xenopus laevis* metallothionein-A. Gene 1995;160:201–206.
30. Palmiter RD. 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 USA 1994;91:1219–1223.
31. Zeng J, Heuchel R, Schaffner W, Kägi JH. Thionein (apometallothionein) can modulate DNA binding and transcription activation by zinc finger containing factor Sp1. FEBS Lett 1994;279:310–312.
32. Chu WA, Moehlenkamp JD, Bittel D, Andrews GK, Johnson JA. Cadmium-mediated activation of the metal response element in human neuroblastoma cells lacking functional metal response element-binding transcription factor-1. J Biol Chem 1999;274:5279–5284.
33. Dalton TP, Li Q, Bittel D, Liang L, Andrews GK. Oxidative stress activates metal-responsive transcription factor-1 binding activity. Occupancy in vivo of metal response elements in the metallothionein-I gene promoter. J Biol Chem 1996;271:26233–26241.
34. Günes C, Heuchel R, Georgiev O, Müller K-H, Lichtlen P, Blüthmann H, Marino S, Aguzzi A, Schaffner W. Embryonic lethality and liver degeneration in mice lacking the metal-responsive transcriptional activator MTF-1. EMBO J 1998;17:2846–2854.
35. Archary MP, Jaggernauth W, Gross E, Alfieri A, Klinger HP, Vikram B. Cell lines from the same cervical carcinoma but with different radiosensitivities exhibit different cDNA microarray patterns of gene expression. Cytogenetics & Cell Genetics 2000;91:39–43.
36. Smirnova IV, Bittel DC, Ravindra R, Jiang H, Andrews GK. Zinc and cadmium can promote rapid nuclear translocation of metal response element-binding transcription factor-1. J Biol Chem 2000;275:9377–9384.
37. Saydam N, Georgiev O, Nakano MY, Greber UF, Schaffner W. Nucleocytoplasmic trafficking of metal-responsive transcription factor MTF-1 is regulated by diverse stress signals. J Biol Chem;276:25487–25495.
38. Vanacore RM, Eskew JD, Morales PJ, Sung I, Smith A. Role for copper in transient oxidation and nuclear translocation of MTF-1, but not NF-kappa B, by the heme-hemopexin transport system. Antioxidants & Redox Signaling 2000;2:739–752.
39. Yu CW, Chen JH, Lin LY. Metal-induced metallothionein gene expression can be inactivated by protein kinase C inhibitors. FEBS Lett 1997;420:69–73.
40. Michalska AE, Choo KHA. Targeting and germ-line transmission of a null mutation at the metallothionein I and II loci in mouse. Proc Natl Acad Sci USA 1993;90:8088–8092.
41. Masters BA, Kelly EJ, Quaife CF, Brinster RL, Palmiter RD. Targeted disruption of metallothionein I and II genes increases sensitivity to cadmium. Proc Natl Acad Sci USA 1994;91:584–588.
42. Andrews GK, Geiser J. Expression of the mouse metallothionein-I and -II genes provides a reproductive advantage during maternal dietary zinc deficiency. J Nutr 1999;129:1643–1648.
43. Erickson JC, Hollopeter G, Thomas SA, Froelick GJ, Palmiter RD. Disruption of the metallothionein-III gene in mice: analysis of brain zinc, behaviour, and neuron vulnerability to metals, aging, and seizures. J Neurosci 1997;17:1271–1281.
44. Hilberg F, Aguzzi A, Howells N, Wagner EF. c-Jun is essential for normal mouse development and hepatogenesis. Nature 1993;365:179–181.
45. Beg AA, Sha WC, Bronson RT, Gosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the relA component of NF-κB. Nature 1995;376:167–170.
46. Bladt F, Reitmacher D, Isenmann S, Aguzzi A, Birchmeier C. Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. Nature 1995;376:768–771.
47. Schmidt C, Bladt F, Goedecke S, Brinkmann V, Zschische W, Sharpe M, Gherardi E, Birchmeier C. Scatter factor/hepatocyte growth factor is essential for liver development. Nature 1995;373:699–702.
48. Uehara Y, Minowa O, Mori C, Shiota K, Kuno J, Noda T, Kitamura N. Placental defect and embryonic lethality in mice lacking hepatocyte growthfactor/scatter factor. Nature 1995;373:702–705.
49. Ganiatsas S, Kwee L, Fujiwara Y, Perkins A, Ikeda T, Labow MA. SEK1 deficiency reveals mitogen-activated protein kinase cascade cross-regulation and leads to abnormal hepatogenesis. Proc Natl Acad Sci USA 1998;95:6881–6886.
50. Derijard B, Hibi M, Wu I, Barrett T, Su B, Deng T, Karin M, Davis RJ. JNK1: a protein kinase stimulated by UV light and Ha-ras that binds and phosphorylates the c-jun activation domain. Cell 1994;76:1025–1035.
51. Sanchez I, Hughes RT, Mayer BJ, Yee K, Woodgett JR, Avruch J. Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-jun. Nature 1994;372:794–798.
52. Rosenfeld ME, Prichard L, Shiojiri N, Fausto N. Prevention of hepatic apoptosis and embryonic lethality in RelA/TNFR-1 double knockout mice. Am J Pathol 2000;156:997–1007.
53. Lichtlen P, Georgiev O, Schaffner W, Aguzzi A, Brandner S. The heavy metal-responsive transcription factor-1 (MTF-1) is not required for neural differentiation. Biol Chem 1999;380:711–715.
54. Fu K, Tomita T, Sarras MP, De Lisle RC, Andrews GK. Metallothionein protects against cerulein-induced acute pancreatitis: analysis using transgenic mice. Pancreas 1998;17:238–246.
55. Quaife CJ, Kelly EJ, Masters BA, Brinster RL, Palmiter RD. Ectopic expression of metallothionein-III causes pancreatic acinar cell necrosis in transgenic mice. Toxicol Appl Pharmacol 1998;148:148–157.
56. Palmiter RD. The elusive function of metallothioneins. Proc Natl Acad Sci USA 1998;95:8428–8430.
57. Meister A. Mitochondrial changes associated with glutathione deficiency. Biochem Biophys Acta 1995;1271:35–42.
58. Bagchi D, Bagchi M, Hassoun EA, Stohs SJ. Cadmium-induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion, and hepatic lipid peroxidation in Sprague-Dowlay rats. Biol Trace Elem Res 1996;52:143–154.
59. Lichtlen P, Wang Y, Belser T, Georgiev O, Certa U, Sack R, Schaffner W. Target gene search for the metal-responsive transcription factor MTF-1. Nucleic Acids Res 2001;29:1514–1523.
60. Timchenko NA, Harris TE, Wilde M, Bilyeu TA, Burgess-Beusse BL, Finegold MJ, Darlington GJ. CCAAT/enhancer binding protein α regulates p21 protein and hepatocyte proliferation in newborn mice. Mol Cell Biol 1997;17:7353–7361.
61. Wang ND, Finegold MJ, Bradley A, Ou CN, Abdelsayed SV, Wilde MD, Taylor LR, Wilson DR, Darlington GJ. Impaired energy homeostasis in C/EBP alpha knockout mice. Science 1995;269:1108–1112.
62. Burgess-Beusse BL, Darlington GJ. C/EBP alpha is critical for the neonatal acute-phase response to inflammation. Mol Cell Biol 1998;18:7269–7277.
63. Mizejewski GJ. Alpha-fetoprotein as a biologic response modifier: relevance todomain and subdomain structure. Proc Soc Exp Biol Med 1997;215:333–362.
64. Langmade SJ, Ravindra R, Daniels PJ, Andrews GK. The transcription factor MTF-1 mediates metal regulation of the mouse ZnT-1 gene. J Biol Chem 2000;275:34803–34809.
65. Jasani B, Schmid KW. Significance of metallothionein overexpression in human tumors. Histopathology 1997;31:211–214.
66. Moussa M, Kloth D, Peers G, Cherian MG, Frei JV, Chin JL. Metallothionein expression in prostatic carcinoma: correlation with Gleason grade, pathologic stage, DNA content and serum level of prostate-specific antigen. Clin Invest Med 1997;20:371–380.
67. Murphy BJ, Andrews GK, Bittel D, Discher DJ, McCue J, Green CJ, Yanovsky M, Giacca A, Sutherland RM, Laderoute KR, Webster KA. Activation of metallothionein gene expression by hypoxia involves metal response elements and metal transcription factor-1. Cancer Res 1999;59:1315–1322.
68. Green CJ, Lichtlen P, Huynh NT, Yanovsky M, Laderoute KR, Schaffner W, Murphy BJ. Placenta growth factor gene expression is induced by hypoxia in fibroblasts: a central role for metal transcription factor MTF-1. Cancer Res 2001;61:2696–2703.
69. Hall PA, Lane DP. Tumour suppressors: A developing role for p53? Curr Biol 1997;7:R144–R147.