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MINI-REVIEW

Involvement of metallothionein and copper in cell proliferation

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Metallothionein is a low-molecular weight, cysteine-rich, metal-binding protein which has been implicated in the detoxification of toxic metals (cadmium, mercury), metabolism of zinc and copper, as well as in the scavenging of free radicals. Recent evidence suggests that the protein may also be involved in cell proliferation. Based on the experiments carried out so far, it is assumed that the fundamental role of metallothionein in cell proliferation may be to detoxify and/or transfer copper ions from the cytoplasm to the nucleus at the G_1/S phase, which in turn participate in some way in nuclear DNA synthesis.

Keywords: cell proliferation, copper, metallothionein

Introduction

Metallothionein (MT) is a metal-binding protein originally isolated from equine renal cortex by Margoshes & Vallee (1957). Later studies revealed that MT occurs throughout the animal kingdom, and is also found in higher plants, eukaryotic microorganisms and some prokaryotes. In animals, the protein is most abundant in parenchymatous tissues, i.e. liver, kidney, pancreas and intestines (Kägi & Schäffer 1988, Robinson 1989, Kay *et al*. 1991).

The characteristic features of MT are its low molecular weight, 6000–7000, and its unusual amino acid composition: cysteine accounts for 30% of the residues and aromatic amino acids are absent. The cysteine residues along the polypeptide chain are fixed and organized into Cys–X–Cys, Cys–Cys or Cys–X–Y–Cys sequences. All cysteinyl-SH groups are involved in ligation of metal ions. Normally MT binds seven atoms of cadmium or zinc per molecule or 12 atoms of copper. A tetrahedral arrangement of four sulfur atoms surrounds each cadmium or zinc atom, while a trigonal arrangement surrounds the copper atoms. The metals are localized in two polynuclear clusters in two distinct domains, each containing half of the polypeptide chain. The C-terminal or α-domain contains four cadmium or zinc atoms, whereas the N-terminal or β-domain contains three atoms of the metals. Each domain also has the potential to bind six copper atoms. Interestingly, copper binding occurs preferentially in the β-domain and cadmium or zinc in the α-domain. Moreover, copper in its cuprous form binds more firmly than cadmium, which in turn binds more firmly than zinc. MT from *Neurospora*, which corresponds to the β-domain of vertebrate MT, as well as yeast MT bind *in vivo* only copper (for review, see Armitage *et al*. 1982, Bremner 1987, Kägi & Schäffer 1988, Bremner & Beattie 1990).

A most remarkable feature of MT is its inducibility. It is induced by a wide range of metals (cadmium, zinc, copper, mercury, bismuth), as well as by specific hormones and other agents including glucocorticoids, catecholamines, glucagon, interleukin-1, interleukin-6, dibutyryl cAMP, phorbol esters, protein kinase C and protein kinase A (Kägi & Schäffer 1988, Garrett *et al*. 1992). The control of MT synthesis occurs at the transcriptional level. The transcription of the MT gene is mediated through specific DNA sequences in the promoter region and transcription factor(s) of protein nature, e.g. ACE1 in *Saccharomyces cerevisiae* (Thiele 1988), and

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MBF-1 (Imbert *et al.* 1989), p39 (Andersen *et al.* 1990), MEP-1 (Labbe *et al.* 1991) and MREBP (Koizumi *et al.* 1992) in mammalian cells. It has been proposed that the transcription factors bind metals, become activated and then bind to specific sequences on the MT gene, thereby increasing transcription (Palmiter 1987, Searle 1987, Fürst *et al.* 1988, Karin *et al.* 1988).

Although MT was discovered 35 years ago, the function specific to the protein still remains a topic of discussion. As far the protein has been implicated in the detoxification of toxic metals (cadmium, mercury), in the metabolism of zinc and copper, as well as in the scavenging of free radicals (Cousins 1985, Richards 1989, Bremner & Beattie 1990). Accumulating evidence suggests that MT may also be involved in cell proliferation, which is the subject of this article.

MT and the cell cycle

The highly conserved protein in evolutionary terms, its ubiquitous occurrence, and the programmed synthesis of MT during tissue regeneration and development (Andrews *et al.* 1991, Nemer *et al.* 1991) are significant arguments for its involvement in some fundamental cellular processes, e.g. the cell cycle. Ohtake *et al.* (1978) have shown, for the first time, the parallelism of enhanced DNA synthesis with increased MT formation in the liver of rats recovering from partial hepatectomy. Later studies, using immunohistochemical methods, have revealed the presence of MT under normal physiological conditions, especially in actively dividing cells, e.g. in various epithelia (Nishimura *et al.* 1989b, 1990, 1991). The authors conclude that MT immunostaining is related mainly to the turnover rate of a tissue, e.g. the relatively short turnover of the cornea epithelium (3–4.5 days) accounts for stronger MT immunostaining in this tissue than in the epidermis in which keratinocytes are replaced with cells migrating from the basal layer with a turnover of 15–30 days (Nishimura *et al.* 1991). In addition, application of tetradecanoyl phorbol ester, a potent cancer promoting and hyperplasia-inducing agent, to mouse skin results in activation of cell divisions in the basal layer (Nishimura *et al.* 1988). Under these conditions both [^{3}H]thymidine was found to be incorporated into cell nuclei and MT immunofluorescence was observed in the dividing basal cells. The intense MT immunostaining has been also demonstrated in spermatogonia and basal cells of seminal vesicles and the ejaculatory duct in rats (Nishimura *et al.* 1990), as well as in human uroepithelium displaying dysplastic changes or carcinoma (Bahnson *et al.* 1991).

Immunohistochemical methods have also provided interesting information on the intracellular distribution of MT. For example, in the rat perinatal liver, which may be used as a model for rapidly dividing cells, there is a gradual increase in levels of cytoplasmic MT during gestation, but at day 20, MT is mainly localized in the nuclei and remains so for several days after birth; thereafter, the intranuclear localization of MT decreases with age (Panemangalore *et al.* 1983). The intense immunostaining of MT is also found in the nuclei of fetal and newborn human liver and kidney (Nartey *et al.* 1987), as well as in the nuclei of leptotene spermatocytes (Nishimura *et al.* 1990). Furthermore, in the partially hepatectomized rat liver, which is used as a model for actively growing tissues, MT is localized predominantly in the nuclei, whereas MT is found only in the cytoplasm of the laparotomized rat liver (Nishimura *et al.* 1989a). To get more insight into the relationship between MT and the cell cycle, Tsujikawa *et al.* (1991) stimulated primary cultured adult rat hepatocytes by epidermal growth factor (EGF) and insulin and then examined by indirect immunofluorescence the intracellular localization of MT. The study revealed that MT staining is confined to the cytoplasm in G_1 phase, while in the early S phase the protein is mainly localized in the cell nuclei. However, in the period of maximal DNA synthesis MT staining in nuclei appears to be weaker than in the cytoplasm. The authors pointed out that the stimulation of proliferation by EGF is necessary for localization of MT in the nuclei of primary cultured hepatocytes. Generally, these studies strongly suggest that intracellular changes in the localization of MT are closely related to cell proliferation, especially to early stages (G_1/S) of this process.

Unfortunately, immunohistochemical methods, although very important in examining the intracellular distribution of MT under normal physiological conditions in particular, have not provided any information on the role of MT in the nucleus and cytoplasm during the cell cycle, as well as whether the nuclear and cytoplasmic MT are present as zinc and/or copper-containing proteins. The answers to these questions, at least in part, have been obtained by using biochemical methods. For instance, Ohtake *et al.* (1978) have found the presence of high amounts of Zn–MT in the cytoplasm of liver in rats recovering from partial hepatectomy. High levels of MT associated mainly with zinc and negligible amounts of copper are also present in the cytoplasm

of fetal and postnatal liver of rats (Mason *et al.* 1980), mouse and Chinese hamster (Bakka & Webb 1981), and humans (Riordan & Richards 1980, Klein *et al.* 1991). On the contrary, the cytoplasmic MT from the perinatal liver of calf (Hartman & Weser 1977), Syrian hamster (Bakka & Webb 1981) and guinea-pig (Lui 1987) contains primarily copper. It is worthy of note, however, that both the metal composition and intracellular localization of MT may undergo essential changes during the course of development. The idea is supported by a recent study (Włostowski 1992) which revealed that the cytoplasmic MT in the newborn liver of bank vole is a zinc-containing protein, the concentration of which abruptly decreases between 1 and 5 days following birth, just in the period directly preceding a rapidly growing liver, but most of the lost cytoplasmic MT is recovered in the nuclei as a copper-containing protein. These changes may be explained by a substitution process involving the pool of Zn–MT already present in the cytoplasm and the increasing concentration of the total and nuclear copper during that period (Włostowski 1992). Since copper has a higher binding affinity for MT than zinc (Day *et al.* 1981, Kägi & Schäffer 1988), one may conclude that copper displaces zinc already bound to MT in the cytoplasm and subsequently the complex Cu–MT is translocated to the nuclei. There is some circumstantial evidence in the literature indicating that it may also be the case in the neonatal liver of rats. Thus an abrupt decline in the cytoplasmic Zn–MT at 4–14 days postpartum observed by Wong & Klaassen (1979) coincides with a sharp increase in the total and nuclear copper concentrations (Evans *et al.* 1970, Terao & Owen 1977). In addition, at the 13th day following birth the cytoplasmic MT contains greater amounts of copper than zinc (Holt *et al.* 1987), suggesting an involvement of the substitution process.

Although more study is needed to confirm the assumption, the aforementioned data suggest that MT may function as a copper transfer protein from cytoplasm to nuclei and/or as a copper detoxifying factor in the cell nuclei. Also, it cannot be excluded that the complex Cu–MT, by itself, is involved in some way in the triggering of the S phase as the protein is localized in nuclei only in the early S phase (Tsujikawa *et al.* 1991). However, if MT is a necessary protein for the onset of S phase, then a lack of this protein should prevent the cell from the proliferation. There is some evidence in the literature indicating that it is not the case, at least for *S. cerevisiae* cells. It has been shown that yeast cells lacking their own MT-like copper-binding protein grow normally under standard laboratory conditions, but are hypersensitive to copper poisoning (Thiele *et al.* 1986). Interestingly, expression of monkey MT complementary DNAs in these cells restored their ability to grow in a high copper medium. Thus it is conceivable that MT plays a similar role in mammalian cells and that the protein is not involved directly in cell proliferation, but indirectly, e.g. through detoxification and/or transfer of copper from cytoplasm to nucleus. This conclusion implies, however, that copper may also play a fundamental role in cell proliferation.

Copper and cell proliferation

The precise role of copper in cell proliferation is as yet unknown, although increasing evidence supports the idea that this trace element may be involved in the cell cycle. For instance, the nuclear copper concentration reaches the highest level (62–71% of the total tissue copper) in the period directly preceding and during a rapidly growing liver in the neonatal bank vole (Włostowski 1992). Likewise, in the human fetal liver, as well as in the newborn rat liver the highest proportion of copper is recovered from the nuclear fraction, whereas over 60% of the total tissue copper is present in the cytosol in the adult liver (Evans *et al.* 1970, Nartey *et al.* 1987). Apart from the perinatal liver exhibiting several-fold higher concentrations of copper than the adult liver (Evans *et al.* 1970, Terao & Owen 1977, Riordan & Richards 1980, Bakka & Webb 1981, Lui 1987, Nartey *et al.* 1987, Klein *et al.* 1991, Włostowski 1992), the cells of malignant tumors display a higher ability to accumulate copper than their normal counterparts (Nederbragt *et al.* 1989). However, if an increase in intracellular copper concentration, especially in the nuclei, is a necessary condition for cell proliferation, then any deficiency of this metal should suppress the process. Indeed, an inhibition of the proliferation, particularly nuclear DNA synthesis, has been found to occur in spleen lymphoid cells (SLC) from copper-deficient rodents (Lukasiewicz & Prohaska 1983, Davis *et al.* 1987, Kramer *et al.* 1988). A more recent study (Padhye *et al.* 1992) has revealed that naphtoquinone thiosemicarbazones—potent chelators of copper—efficiently inhibit DNA synthesis in P388 lymphocyte cells. Although thiosemicarbazones which strongly bind copper may have directly limited the availability of the metal for the cells in the latter case, in the former one the limitation of copper for SLC may have resulted from an accompanied deficiency of ceruloplasmin activity in the blood plasma (Kramer *et al.* 1988) as this

glycoprotein, synthesized in the liver, is thought to be a major copper donor to extrahepatic tissues (Cousins 1985). If this is the case, then one may assume also that the need for greater amounts of copper, e.g. for the proliferation of cells participating in immune response, may account for an elevation of ceruloplasmin and copper levels in the plasma observed during inflammation and infection (Laurin & Klasing 1987, Klasing 1988, Richards & Augustine 1988). Furthermore, it is noteworthy that cytokines (e.g. interleukin-1) released by activated macrophages induce in the liver both ceruloplasmin and MT (Klasing 1988, Barber & Cousins 1988). In addition, cytokines probably induce the synthesis of MT also in lymphocytes (Oberbarnscheidt *et al.* 1988), in which the regulatory mechanism of MT production seems to be similar to that in the liver (Yamada & Koizumi 1991). Thus it cannot be excluded that MT, ceruloplasmin and copper are involved, among other things, in the lymphocyte proliferation during immune response.

Further circumstantial evidence suggesting an involvement of ceruloplasmin in copper delivery to dividing cells comes from studies on copper metabolism during and after pregnancy in mammals (Terao & Owen 1977). For instance, maternal plasma ceruloplasmin and copper concentrations increase during pregnancy but decrease postpartum. Simultaneously, biliary excretion of copper almost stops at term and remains decreased thereafter. In addition, the content of copper in the newborns, especially in the liver, is very high. These data strongly suggest that the mother stores copper for the use of her fetuses and newborn pups, and that ceruloplasmin transfers copper directly (fetuses) or indirectly (through milk) to the rapidly growing offspring. Otherwise their growth and development can be significantly disturbed. This situation exists, e.g. for dams producing milk deficient in copper, which bear an autosomal mutation referred to as toxic milk (Rauch 1983). Thus, as in the case of an inflammatory response, it is reasonable to assume that high amounts of copper being transferred on ceruloplasmin from mother to offspring may also be implicated in the process of cell division.

Concluding remarks

The literature data presented in this paper strongly suggest that MT and copper play important functions in the cell cycle, although their precise role still remains to be established. Nevertheless, based on the experiments carried out to date, one may conclude that the fundamental role of MT in cell proliferation may be to detoxify and/or transfer copper ions from the cytoplasm to the nucleus at the G_1/S phase, which in turn may participate in some way in the nuclear DNA synthesis as the process is accompanied by very high levels of copper in the cell nuclei and any deficiency of copper suppresses the incorporation of [^{3}H]thymidine. Further studies are urgently needed, however, to verify the conclusion since it is based mainly on indirect inference.

References

Andersen RD, Taplitz SJ, Oberbauer AM, Calome KL, Herschman HR. 1990 Metal-dependent binding of nuclear factor to the rat metallothionein-I promoter. *Nucl Acids Res* **18**, 6049–6055.

Andrews GK, Huet-Hudson YM, Paria BC, McMaster MT, De SK, Dey SK. 1991 Metallothionein gene expression and metal regulation during preimplantation mouse embryo development (MT mRNA during early development). *Dev Biol* **145**, 13–27.

Armitage IM, Otvos JD, Briggs RW, Boulanger Y. 1982 Structure elucidation of the metal-binding sites in metallothionein by ^{113}Cd NMR. *Fed Proc* **41**, 2974–2980.

Bahnson RR, Banner BF, Ernstoff MS, *et al.* 1991 Immunohistochemical localization of metallothionein in transitional cell carcinoma of the bladder. *J Urol* **146**, 1518–1520.

Bakka A, Webb M. 1981 Metabolism of zinc and copper in the neonates: changes in the concentrations and contents of thionein-bound Zn and Cu with age in the liver of the newborn of various mammalian species. *Biochem Pharmacol* **30**, 721–725.

Barber EF, Cousins RJ. 1988 Interleukin-1-stimulated induction of ceruloplasmin synthesis in normal and copper-deficient rats. *J Nutr* **118**, 375–381.

Bremner I. 1987 Involvement of metallothionein in the hepatic metabolism of copper. *J Nutr* **117**, 19–27.

Bremner I, Beattie JH. 1990 Metallothionein and the trace minerals. *Annu Rev Nutr* **10**, 63–83.

Cousins RJ. 1985 Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol Rev* **65**, 238–309.

Davis MA, Johnson WT, Briske-Anderson M, Kramer TR. 1987 Lymphoid cell functions during copper deficiency. *Nutr Res* **7**, 211–222.

Day FA, Panemangalore M, Brady FO. 1981 *In vivo* and *ex vivo* effect of copper on rat liver metallothionein. *Proc Soc Exp Biol Med* **168**, 306–310.

Evans GW, Myron DR, Cornatzer NF, Cornatzer WE. 1970 Age-dependent alteration in hepatic subcellular copper distribution and plasma ceruloplasmin. *Am J Physiol* **218**, 298–302.

Fürst P, Hu S, Hackett R, Hamer D. 1988 Copper

activates metallothionein gene transcription by altering the conformation of a specific DNA binding protein. *Cell* **55**, 705–717.

Garrett SH, Xiong X, Arizono K, Brady FO. 1992 Phorbol ester induction of rat hepatic metallothionein *in vivo* and *in vitro*. *Int J Biochem* **24**, 1669–1676.

Hartmann HJ, Weser U. 1977 Copper-thionein from fetal bovine liver. *Biochim Biophys Acta* **155**, 631–635.

Holt D, Snowden R, Dinsdale D, Webb M. 1987 Intestinal uptake and retention of copper in the suckling rat. *Rattus rattus*—IV. Mechanisms of intestinal copper accumulation. *Comp Biochem Physiol* **86C**, 191–200.

Imbert J, Zafarullah M, Culotta VC, Gedamu L, Hamer D. 1989 Transcription factor MBF-I interacts with metal regulatory elements of higher eucaryotic metallothionein genes. *Mol Cell Biol* **9**, 5315–5323.

Kägi JHR, Schäffer A. 1988 Biochemistry of metallothionein. *Biochemistry* **27**, 8509–8515.

Karin M, Imagawa M, Bodner M, *et al*. 1988 Regulation of human metallothionein and growth hormone gene expression by steriod hormone receptors and other *trans*-acting factors. In: Ringold GH, ed. *Steroid Hormone Action, UCLA Symposia on Molecular and Cellular Biology*, Vol. 75. New York: AR Liss; 177–184.

Kay J, Cryer A, Darke B, *et al*. 1991 Naturally occurring and recombinant metallothioneins: structure, immunoreactivity and metal-binding functions. *Int J Biochem* **23**, 1–5.

Klasing KC. 1988 Nutritional aspects of leukocytic cytokines. *J Nutr* **118**, 1436–1446.

Klein D, Scholz P, Drasch GA, Müller-Höcker J, Summer KH. 1991 Metallothionein, copper and zinc in fetal and neonatal human liver: changes during development. *Toxicol Lett* **56**, 61–67.

Koizumi S, Suzuki K, Otsuka F. 1992 A nuclear factor that recognizes the metal-responsive elements of human metallothionein II_A gene. *J Biol Chem* **267**, 18659–18664.

Kramer TR, Johnson WT, Briske-Anderson M. 1988 Influence on iron and the sex of rats on hematological, biochemical and immunological changes during copper deficiency. *J Nutr* **118**, 214–221.

Labbe S, Prevost J, Remondelli P, Leone A, Seguin C. 1991 A nuclear factor binds to the metal regulatory elements of the mouse gene encoding metallothionein-I. *Nucl Acids Res* **19**, 4225–4231.

Laurin DE, Klasing KC. 1987 Effects of repetitive immunogen injections and fasting versus feeding on iron, zinc, and copper metabolism in chicks. *Biol Trace Elem Res* **14**, 153–165.

Lui EMK. 1987 Metabolism of copper and zinc in the liver and bone of the perinatal guinea-pig. *Comp Biochem Physiol* **86C**, 173–183.

Lukasiewicz OA, Prohaska JR. 1983 Lymphocytes from copper-deficient mice exhibit decreased mitogen reactivity. *Nutr Res* **3**, 335–341.

Margoshes M, Vallee BL. 1957 A cadmium protein from equine kidney cortex. *J Am Chem Soc* **79**, 4813–4814.

Mason R, Bakka A, Samarawickrama GP, Webb M. 1980 Metabolism of zinc and copper in the neonates: accumulation and function of (Zn, Cu)-metallothionein in the liver of the newborn rat. *Br J Nutr* **45**, 375–389.

Nartey NO, Banerjee D, Cherian MG. 1987 Immunohistochemical localization of metallothionein in cell nucleus and cytoplasm of fetal human liver and kidney and its changes during development. *Pathology* **19**, 233–238.

Nederbragt H, de Poel WJE, Toussaint MJM, de Witt MML. 1989 Influence of differentiation status of cells on the metabolism of copper and zinc *in vitro*. In: Anke M, Baumann W, Bräunlich H, Brüchner C, Groppel B, Grün M. eds. *6th Int Trace Element Symp*, Vol. 2. Leipzig/Jena: Universitätsverlag; 562–568.

Nemer M, Thornton RD, Stuebing EW, Harlow P. 1991 Structure, spatial, and temporal expression of two sea urchin metallothionein genes, $SpMTB_1$ and SpMTA. *J Biol Chem* **266**, 6586–6593.

Nishimura N, Nishimura H, Tohyama C, Karasawa M, Kuroki T. 1988 Localization of metallothionein in epidermal hyperplasia caused by topical application of tumor promotor, 12-*O*-tetradecanoylphorbol-13-acetate, on the mouse skin. *J Histochem Cytochem* **36**, 945.

Nishimura H, Nishimura N, Tohyama C. 1989a Immunohistochemical localization of metallothionein in developing rat tissues. *J Histochem Cytochem* **37**, 715–722.

Nishimura N, Nishimura H, Tohyama C. 1989b Localization of metallothionein in female reproductive organs of rat and guinea-pig. *J Histochem Cytochem* **37**, 1601–1607.

Nishimura H, Nishimura N, Tohyama C. 1990 Localization of metallothionein in the genital organs of the male rat. *J Histochem Cytochem* **38**, 927–933.

Nishimura H, Nishimura N, Kobayaski S, Tohyama C. 1991 Immunohistochemical localization of metallothionein in the eye of rats. *Histochemistry* **95**, 535–539.

Oberbarnscheidt J, Kind P, Abel J, Gleichmann E. 1988 Metallothionein induction in a human B cell line by stimulated immune cell products. *Res Commun Chem Pathol Pharmacol* **60**, 211–224.

Ohtake H, Hasegawa K, Koga M. 1978 Zinc-binding protein in the livers of neonatal, normal and partially hepatectomized rats. *Biochem J* **174**, 999–1005.

Padhye S, Chikate R, Kumbhar A, Shallom JM, Chitnis MP. 1992 Novel quinone–thiosemicarbazone hybrid (QTSCHY) non-platinum antitumor agents—inhibition of DNA biosynthesis in P388 lymphocyte cells by coordinatively unsaturated copper (II) and iron (III) complexes of naphtoquinone thiosemicarbazones. *BioMetals* **5**, 67–72.

Palmiter RD. 1987 Molecular biology of metallothionein gene expression. *Experientia* **52** (Suppl), 63–80.

Panemangalore M, Banerjee D, Onosaka S, Cherian MG. 1983 Changes in intracellular accumulation and distribution of metallothionein in rat liver and kidney during postnatal development. *Dev Biol* **97**, 95–102.

Rauch H. 1983 Toxic milk, a new mutation affecting copper metabolism in the mouse. *J Hered* **74**, 141–144.

Richards MP. 1989 Recent developments in trace element

metabolism and function: role of metallothionein in copper and zinc metabolism. *J Nutr* **119**, 1062–1070.

Richards MP, Augustine PC. 1988 Serum and liver zinc, copper, and iron in chicks infected with *Eimeria acervulina* and *Eimeria tenela*. *Biol Trace Elem Res* **17**, 207–219.

Riordan JR, Richards V. 1980 Human fetal liver contains both zinc- and copper-rich forms of metallothionein. *J Biol Chem* **255**, 5380–5383.

Robinson NJ. 1989 Algal metallothioneins: secondary metabolites and proteins. *J Appl Phycol* **1**, 5–18.

Searle PF. 1987 Metallothionein gene regulation. *Biochem Soc Trans* **15**, 584–586.

Terao T, Owen GA. 1977 Copper metabolism in pregnant and postpartum rat and pups. *Am J Physiol* **232**, E172–E179.

Thiele DJ. 1988 ACE1 regulates expression of the *Saccharomyces cerevisiae* metallothionein gene. *Mol Cell Biol* **8**, 2745–2752.

Thiele DJ, Walling MJ, Hamer DH. 1986 Mammalian metallothionein is functional in yeast. *Science* **231**, 854–856.

Tsujikawa K, Imai T, Kakutani M, *et al*. 1991 Localization of metallothionein in nuclei of growing primary cultured adult rat hepatocytes. *FEBS Lett* **283**, 239–242.

Włostowski T. 1992 Postnatal changes in subcellular distribution of copper, zinc and metallothionein in the liver of bank vole (*Clethrionomys glareolus*): a possible involvement of metallothionein and copper in cell proliferation. *Comp Biochem Physiol* **1030**, 285–290.

Wong KL, Klaassen CD. 1979 Isolation and characterization of metallothionein which is highly concentrated in newborn rat liver. *J Biol Chem* **254**, 12399–12403.

Yamada H, Koizumi S. 1991 Metallothionein induction in human peripherial blood lymphocytes by heavy metals. *Chem-Biol Interact* **78**, 347–354.