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Perspective

The elusive function of metallothioneins

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ABSTRACT **Biochemistry and genetics are both required to elucidate the function of macromolecules. There is no question that metallothioneins (MTs) have unique biochemical properties, but genetic experiments have not substantiated the importance of MTs under physiological conditions. Even after thousands of studies describing the structure, biochemical characteristics, tissue distribution, induction, and consequences of genetic disruption and deliberate overexpression, the evolutionary forces that led to the initial appearance, gene duplications, and nearly ubiquitous expression of MTs remain enigmatic.**

Genes encoding the small, metal-binding metallothioneins (MTs) are found in all eukaryotes (often in multiple copies) as well as some prokaryotes (1). MTs are unusually rich in cysteine residues that coordinate multiple zinc and copper atoms under physiological conditions. In the mouse, there are four MT genes that reside in a 50-kb region on chromosome 8, whereas humans have at least 16 MT genes clustered on chromosome 16 (2, 3). The mouse MT I and II genes are expressed at all stages of development in many cell types of most organs; they are coordinately regulated by metals, glucocorticoids, and inflammatory stress signals (4). MT III is expressed predominantly in neurons but also in glia and male reproductive organs (5–7). MT IV is expressed in differentiating stratified squamous epithelial cells (3). All four MT genes are expressed in the maternal deciduum (8). The fact that there are multiple MT genes, expressed in distinct patterns, suggests that they should have important functions; however, whether they have redundant or divergent functions (9) is not yet clear.

In single-cell eukaryotes, MTs bind copper predominantly (10, 11). Mutations that prevent MT synthesis confer copper sensitivity, whereas excess expression of MTs confers resistance to copper toxicity (10, 12). In mammals, MTs bind zinc predominantly, but zinc can be readily displaced by copper or cadmium (13). Mice (14, 15) and various mammalian cell lines (4, 16, 17) that cannot synthesize any MT are sensitive to cadmium toxicity, whereas mice (18) and cells (19–22) that express excess amounts of any MT are resistant to this metal. Indeed, selection for cadmium resistance with mammalian cells invariably results in up to 80-fold amplification of the entire MT locus (23). These observations followed naturally from the original discovery of cadmium-MT in horse kidney by Margoshes and Vallee (24) in 1957. Although a characteristic phenotype of cells and mice with altered expression of MTs is the sensitivity to cadmium toxicity, it seems unlikely that the evolutionary conservation of these ubiquitous, inducible genes in most organisms is driven by the ability of MTs to detoxify cadmium. Although cases of cadmium toxicity are known, they are rare and exclusively caused by man. Thus, it seems more likely that cadmium detoxification is a property of MTs rather than its evolutionary function (25).

It seems more likely that the function of MTs in mammals and other organisms would relate to physiologically relevant metals such as zinc or copper (25). An attractive idea that has received recent support is that MTs might function as chaperones for synthesis of metalloproteins. MTs could serve as reservoirs of essential metals while preventing metal toxicity and yet donate the metals to apometalloproteins as they are synthesized, or afterward. Experiments *in vitro* indicate that such reactions are possible (26–28), and glutathione has been shown to facilitate such interactions (29, 30). Furthermore, the reactions are reversible: apo-MT can extract zinc from metalloproteins *in vitro* and when injected into *Xenopus* eggs (31, 32).

The multiple cysteine residues of MT can be oxidized, releasing bound metal in the process. In a recent *Proceedings* Maret and Vallee (33) pointed out that the clusters of sulfurs that bind zinc in mammalian MTs create an oxidoreductive environment for zinc at a redox potential so low that MT can be readily oxidized by mild cellular oxidants, such as disulfides, with the release of zinc. They showed that oxidation of MT by mixtures of oxidized and reduced glutathione can readily release zinc from MT *in vitro*, but demonstrating the occurrence of these reactions *in vivo* is a challenging problem. Maret and Vallee (33) "believe that MT has specific redox properties for a purpose that selectively controls the release and uptake of zinc rather than being a nonspecific antioxidant that releases the metal randomly and sporadically." According to this view, the release of zinc from MT, perhaps in response to local changes in redox potential, is an important function of MT.

However, genetic experiments indicate that transfer of copper or zinc from MT to other molecules is not essential. Yeast and mammalian tissue culture cells that cannot make any MT grow in normal medium as well as cells with MT, arguing that all essential metalloproteins can be synthesized without the aide of MT chaperones (4, 10). Furthermore, zinc exchange occurs readily without MT. More than half of the total cellular zinc, most of which is bound to metalloproteins, can be exchanged with zinc in the extracellular medium in a few hours by mammalian cells lacking MT (34) and restoration of MT has little effect on exchange rates (unpublished observations). The most telling experiment that argues against MT playing an important role in zinc transfer reactions is that mice that cannot synthesize either MT I or MT II grow and reproduce normally. These two MTs appear to be the only MTs synthesized by most cells of the mouse. Thus, none of the multitude of cells in which these MTs are made require them for synthesis, function, or regulation of any essential metalloprotein or other zinc-demanding process under normal conditions (14, 15). Perhaps under normal conditions MTs are not necessary, but they could serve as an important reservoir

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Abbreviation: MT, metallothionein.

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of metal that can be tapped when metals are limiting or when cells are exposed to changes in oxidation state.

The glucocorticoid-mediated induction of MT I and MT II in fetal liver during the latter part of gestation results in accumulation of large amounts of zinc- and copper-MT (35). The hepatic concentration of these metals declines after birth, perhaps as a consequence of liver growth or distribution of the metals to other organs. Mice that cannot make MT I and MT II have a low hepatic zinc content at birth, which might be expected to jeopardize perinatal development. However, the only morphological abnormalities observed were in the developing kidney (36). Rearing MT-deficient and control pups on dams fed a zinc-deficient diet stunted the growth of both groups identically. This treatment exacerbated the kidney anomalies of MT-deficient mice but did not appear to impair kidney function. Transgenic mice that overexpress MT I accumulate zinc in several organs, especially the pancreas. Fetuses of pregnant dams that have excess Zn-MT are more resistant to teratogenic and embryotoxic effects of dietary zinc deficiency during pregnancy (37). These genetic experiments indicate that MT I and MT II are not essential for normal growth, but they do provide a reservoir of zinc that can be mobilized under zinc-limiting conditions.

If mammalian MTs are not essential for sequestering and transferring metals required for cell growth and development, then maybe they protect against toxicity of essential metals. Indeed, cells that cannot synthesize any MT and mice that cannot synthesize MTs I and II have marginally increased sensitivity to zinc toxicity (36, 38). Nevertheless, mice lacking both MTs I and II can thrive on water supplemented with 25 mM zinc and only massive doses of injected zinc had deleterious effects, with the pancreas incurring the most damage (36). Similar conclusions were drawn from attempts to demonstrate increased sensitivity to copper toxicity (36, 38). It is true, however, that cells and mice that lack functional zinc (ref. 34; unpublished observations) or copper (39) efflux transporters rely on MT for protection against metal toxicity. Thus, the first line of defense against an influx of heavy metals appears to be rapid metal efflux. Induction of MT and sequestration of these metals by MT provides a secondary line of defense. Cadmium detoxification may rely on MTs because there is no effective efflux system for this metal in mammals.

The discovery of MTs with more restricted expression (MT III and MT IV) suggested that these isoforms have distinct functions, which might shed light on the functions of the more widely expressed isoforms. Indeed, MT III was discovered during an evaluation of the ability of brain extracts from people with Alzheimer disease to support survival of rat neurons in culture (6). Surprisingly, Alzheimer brain extracts supported neuronal survival better than extracts from unaffected brains. An inhibitory activity (initially called GIF, growth inhibitory factor) was isolated from normal brain extracts that turned out to be MT III, and the initial results indicated that this protein was deficient in persons with Alzheimer disease (6). The selective inhibitory effects of MT III on neuron survival have been reproduced (40), and the structural requirements for this effect have been examined (41); however, the association of MT III with Alzheimer disease has not been confirmed (40). Furthermore, mice lacking MT III do not reveal any neurological or behavioral deficiencies, even when 2 years old. But they do manifest increased sensitivity to kainate-induced seizures and greater neuronal damage resulting from such seizures (42). MT III has metal-binding properties similar to conventional MTs (41), but it has biological properties that are distinct from those of MT I when expressed ectopically in cells and mice. When MT III is expressed from a constitutive promoter in BHK cells, it competes for available zinc, whereas MT I does not (38). If MT III is expressed in most organs of transgenic mice with regulatory elements from the MT I and MT II locus, then the mice die as a result of pancreatic acinar cell necrosis (43). Similar levels of MT I expression in pancreas have no effect. Although these results indicate that MT isoforms do have different biological properties, they have not yet revealed what the physiological functions may be.

Another idea that is growing in popularity is that MTs can protect against oxidative damage (44, 45). As indicated above, the metal-thiolate clusters are readily oxidized *in vitro*; thus, they could scavenge deleterious oxygen radicals. Compelling genetic evidence for this concept comes from work with yeast. Yeast that cannot synthesize copper–MTs are more sensitive to oxidative stress if they also lack superoxide dismutase, suggesting that yeast MT has antioxidant functions (46). Expression of monkey MTs under control of yeast MT promoter also protects against oxidative stress (46). These authors also demonstrated that copper–MT was a more effective antioxidant in yeast extracts than was zinc–MT. Reactive oxygen species are generated by leukocytes when they are stimulated by interleukins and interferons that are released in response to lipopolysaccharide. These reactive oxygen species are cytotoxic to invading microorganisms, but they are also potentially harmful to the host. Consequently, the induction of MT I and MT II by these cytokines may reflect a mechanism of protecting the host tissues against oxidative damage (47). Many other agents that induce oxidative stress, such as chloroform, turpentine, diethyl maleate, paraquat, and H_20_2, can induce MT I and II in cells and *in vivo* (48–51). Thus, there is ample reason to suspect that MT might be involved in protecting against oxidative damage. Mammalian cells that express excess MTs appear to be resistant to toxic effects of nitric oxide (52) and many electrophilic antineoplastic agents (53), which are capable of reacting with the cysteines of MT. A number of presentations at the IVth International Metallothionein Meeting last fall (54) described results in which mice or cells that could not synthesize MT were more sensitive to conditions in which oxidative stress is suspected, whereas other investigators found conditions in which overexpression of MT protected against treatments thought to produce oxidative damage. Most of these results have not yet been published or replicated; therefore, it is not yet possible to evaluate the physiological significance of these tantalizing observations. In these cases, it will be important to demonstrate that MTs actually become oxidized *in vivo* and that they protect cells from oxidative insults.

A recent study published in *Proceedings* indicated that mice lacking both MT I and II become obese (55), but the mechanism by which MT affects energy metabolism was not defined. However, these studies suffer from the inappropriate choice of control mice. When mice of identical genetic background (except for the MT I and II locus) were compared at several different laboratories, MT expression had no effect on body weight.

It is surprising that elucidating the function of these unusual, inducible proteins that are expressed ubiquitously should be so difficult. One would think that the distinct biochemistry of these proteins, the nature of the inducers, and the phenotype of mutant cells and animals lacking these proteins would provide invaluable clues. It seems likely that there will be some condition faced by most organisms where these proteins provide a selective advantage, but such a condition has not yet been discovered. If understanding the functions of MTs can be so perplexing, the age of functional genomics will be challenging.

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1. Kägi, J. H. R. (1993) in *Metallothionein III*, eds. Suzuki, K. Y., Imura, N. & Kimura, M. (Birkhäuser, Basel), pp. 29–55.

2. West, A. K., Hildebrand, C. E., Karin, M. & Richards, R. I. (1990) *Genomics* **8,** 513–518.
3. Quaife, C. J., Findley, S. D., Erickson, J. C., Froelick, G. J., Kelly, E. J., Zambrowicz, B. P. & Palmiter, R. D. (1994) *Biochemistry* **33,** 7250–7259.
4. Palmiter, R. D. (1987) *Experientia* **52,** Suppl., 63–80.
5. Masters, B. A., Quaife, C. J., Erickson, J. C., Kelly, E. J., Froelick, G. J., Zambrowicz, B. P., Brinster, R. L. & Palmiter, R. D. (1994) *J. Neurosci.* **14,** 5844–5857.
6. Uchida, Y., Takio, K., Titani, K., Ihara, Y. & Tomonaga, M. (1991) *Neuron* **7,** 3337–3347.
7. Moffatt, P. & Seguin, C. (1998) *DNA Cell Biol.* **17,** 501–510.
8. Liang, L., Fu, K., Lee, D. K., Sobieski R. J., Dalton, T. & Andrews G. K. (1996) *Mol. Reprod. Dev.* **43,** 25–37.
9. Thomas, J. H. (1993) *Trends Genet.* **9,** 395–399.
10. Hamer, D. H. (1986) *Annu. Rev. Biochem.* **55,** 913–951.
11. Winge, D. R & Dameron, C. T. (1993) in *Metallothionein III*, eds. Suzuki, K. Y., Imura, N. & Kimura, M. (Birkhäuser, Basel), pp. 381–397.
12. Thiele, D. J. (1992) *Nucleic Acids Res.* **20,** 1183–1191.
13. Shaw, C. F., III, Savas, M. M. & Petering, D. H. (1991) *Methods Enzymol.* **205,** 401–414.
14. Michalska, A. E. & Choo, A. K. H. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8088–8092.
15. Masters, B. A., Kelly, E. J., Quaife, C. J., Brinster, R. L. & Palmiter, R. D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 584–588.
16. Compere, S. J. & Palmiter, R. D. (1981) *Cell* **25,** 233–240.
17. Palmiter, R. D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1219–1223.
18. Liu, Y., Liu, J., Iszard, M. B., Andrews, G. K., Palmiter, R. D. & Klaassen, C. D. (1995) *Toxicol. Appl. Pharmacol.* **135,** 222–228.
19. Rugstad, H. E. & Norseth, T. (1978) *Biochem. Pharmacol.* **27,** 647–650.
20. Hildebrand, C. E., Tobey, R. A., Campbell, E. W. & Enger, M. D. (1979) *Exp. Cell Res.* **124,** 237–246.
21. Beach, L. R. & Palmiter, R. D. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2110–2114.
22. Karin, M., Cathala, G. & Nguyen-Huu, M. C. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4040–4044.
23. Durnam, D. M. & Palmiter, R. D. (1987) *Experientia* **52,** Suppl., 457–463.
24. Margoshes, M. & Vallee, B. L. (1957) *J. Am. Chem. Soc.* **79,** 4813–4814.
25. Vallee, B. L. (1987) *Experientia* **52,** Suppl., 5–16.
26. Udom, A. O. & Brady, F. O. (1980) *Biochem. J.* **187,** 329–335.
27. Cano-Gauci, D. F. & Sarkar, B. (1996) *FEBS Lett.* **386,** 1–4.
28. Maret, W., Larsen, K. S. & Vallee, B. L. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 2233–2237.
29. Maret, W. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 237–241.
30. Jiang, L.-J., Maret, W. & Vallee, B. L. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 3483–3488.
31. Zeng, J., Vallee, B. L. & Kägi, J. H. R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9984–9988.
32. Jacob, C., Maret, W. & Vallee, B. L. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 3489–3494.
33. Maret, W. & Vallee, B. L. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 3478–3482.
34. Palmiter, R. D. & Findley, S. (1995) *EMBO J.* **14,** 639–649.
35. Quaife, C. J., Hammer, R. E., Mottet, K. & Palmiter, R. D. (1986) *Dev. Biol.* **118,** 549–555.
36. Kelly, E. J., Quaife, C. J., Froelick, G. J. & Palmiter, R. D. (1996) *J. Nutr.* **126,** 1782–1790.
37. Dalton, T., Fu, K., Palmiter, R. D. & Andrews, G. K. (1996) *J. Nutr.* **126,** 825–833.
38. Palmiter, R. D. (1995) *Toxicol. Appl. Pharmacol.* **135,** 139–146.
39. Kelly, E. J. & Palmiter, R. D. (1996) *Nat. Genet.* **13,** 219–222.
40. Erickson, J. C., Sewell, A. K., Jensen, L. T., Winge, D. R. & Palmiter, R. D. (1994) *Brain Res.* **649,** 247–253.
41. Sewell, A. K., Jensen, L. T., Erickson, J. C., Palmtier, R. D. & Winge, D. R. (1995) *Biochemistry* **34,** 4740–4747.
42. Erickson, J. C., Hollopeter, G., Thomas, S. A., Froelick, G. J. & Palmiter, R. D. (1997) *J. Neurosci.* **15,** 1271–1281.
43. Quaife, C. J., Kelly, E. J., Masters, B. A., Brinster, R. L. & Palmiter, R. D. (1998) *Toxicol. Appl. Pharmacol.* **148,** 148–157.
44. Thornalley, P. J. & Vasak, M. (1985) *Biochim. Biophys. Acta* **827,** 36–44.
45. Karin, M. (1985) *Cell* **41,** 8–10.
46. Tamai, K. T., Gralla, E. B., Ellerby, L. M., Valentine, J. S. & Thiele, D. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8013–8017.
47. De, S. K., McMaster, M. T. & Andrews, G. K. (1990) *J. Biol. Chem.* **265,** 15267–15274.
48. Min, K.-S., Terano, Y., Onosaka, S. & Tanaka, K. (1991) *Toxicol. Appl. Pharmacol.* **111**, 152–162.
49. Bauman, J. W., Madhu, C., McKim, J. M., Jr., Liu, Y. & Klaassen, C. D. (1992) *Toxicol. Appl. Pharmacol.* **117,** 233–241.
50. Dalton, T., Palmiter, R. D. & Andrews, G. K. (1994) *Nucleic Acids. Res.* **22,** 5016–5023.
51. Sato, M., Sasaki, M & Hojo, H. (1993) in *Metallothionein III*, eds. Suzuki, K. Y., Imura, N. & Kimura, M. (Birkhäuser, Basel), pp. 125–140.
52. Schwarz, M. A., Lazo, J. S., Yalowich, J. C., Allen, W. P., Whitmore, M., Bergonia, H. A., Tzeng, E., Billar, T. R., Robbins, P. D., Lancaster, J. R., Jr., & Pitt, B. R. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4452–4456.
53. Lazo, J. S., Yang, Y.-Y., Woo, E., Kuo, S.-M. & Saijo, N. (1993) in *Metallothionein III*, eds. Suzuki, K. Y., Imura, N. & Kimura, M. (Birkhäuser, Basel), pp. 293–314.
54. Klaassen, C. D., ed. (1998) *Metallothionein IV* (Birkhäuser, Basel), in press.
55. Beattie, J. H., Wood, A. M., Newman, A. M., Bremner, I., Choo, K. H. A., Michalska, A. E., Duncan, J. S. & Trayhurn, P. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 358–363.