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Biochemistry of Metallothionein[†,‡]

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The proficiency of living systems to cope with reactive heavy metal ions in the environment and to utilize some of them as key constituents in metabolism and growth is an essential achievement of evolution. An important conceptual contribution of biochemistry to our understanding of these faculties was the realization that the biological functions of metals and their passage through cells and organisms are invariably linked to the existence of specific metal-binding macromolecules (Vallee, 1960). Thus, metals occur as components of numerous enzymes, and there are also many nonenzymic metalloproteins and other metal-binding biopolymers serving regulatory purposes or controlling the metabolism of essential and nonessential metal ions themselves.

The mode of metal binding in metalloproteins varies widely, yielding structures of divergent chemical and biological specificity. A particularly active area of research is proteins and polypeptides containing sets of metal ions arranged in oligonuclear complexes, i.e., metal clusters. Classical examples are the iron–sulfur proteins of electron transport systems where two, three, or four iron ions are bound to a combination of thiolate ligands of Cys and of acid-labile inorganic sulfide yielding mixed-ligand clusters. Recently, a novel kind of sulfur-based metal clusters has come to attention in the metallothioneins (MTs) and is now being recognized as the major structural principle of this group of ubiquitous proteins or polypeptides. In these compounds, which preferentially contain sets of d^{10} metal ions, clusters are usually formed by their exclusive coordination to arrays of closely spaced Cys thiolate groups. The present review highlights the chemical and biochemical features of this superfamily of metal-binding macromolecules.

MT was discovered in 1957 when Margoshes and Vallee identified in equine kidney cortex a cadmium-binding protein responsible for the natural accumulation of cadmium in this tissue (Margoshes & Vallee, 1957; Kägi & Vallee, 1960). MTs are still the only biological compounds known to naturally contain this metal. However, as already shown in the earliest studies, cadmium is only one of several optional metallic components, the others being most commonly zinc and copper (Kägi & Vallee, 1961; Pulido et al., 1966). Thus, at least in mammals, MTs are implicated to play roles in connection with a variety of essential and nonessential post-transition-metal ions.

Definition and Occurrence. MTs have received their designation from their extremely high metal and sulfur content that, varying with the metal species present, together may contribute to over 20% of their weight. The mammalian forms are characterized by a molecular weight of 6000–7000, containing some 60 amino acid residues, among them 20 Cys, and binding a total of 7 equiv of bivalent metal ions. Aromatic amino acid residues are absent. All Cys occur in the reduced form and are coordinated to the metal ions through mercaptide bonds, giving rise to spectroscopic features characteristic of metal–thiolate complexes and metal–thiolate clusters. According to recommendations made by The Committee on the Nomenclature of MT, any protein or polypeptide resembling mammalian MTs in several of these criteria can be classified as an MT (Fowler et al., 1987).

MTs occur throughout the animal kingdom and are also found in higher plants, eukaryotic microorganisms, and some prokaryotes [cited in Hamer (1986) and Kägi and Kojima (1987)]. In animals, the protein is most abundant in parenchymatous tissues, i.e., liver, kidney, pancreas, and intestines. There are wide variations in concentration in different species and tissues, reflecting effects of age, stage of development, dietary regimen, and other not yet fully identified factors. In experimental animals and in cultured cells, concentrations can be raised by exposure to a variety of inducing agents (see below). Although MT is a cytoplasmic protein, it can also accumulate in lysosomes (Johnson et al., 1981), and during development it has been observed in the nucleus (Nartey et al., 1987).

Classification and Polymorphism. Taking into account structural relationships, MTs have been subdivided into three classes (Fowler et al., 1987). Class I includes mammalian MTs and polypeptides from other phyla with related primary

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Class I		
	1[b] 20 40 60	
Human (MT-2)[c]	MDP NCSCAAGDSCTCAGSCKCKECKCTSCKKSCCSCCPVGCAKCAQGCICKGASD KCSCCA	Kissling & Kägi, 1977
Chicken	MDPQDCTCAAGDSCSCAGSCKCKNCRCRSCRKSCCSCCPAGCNNCAKGCVCKEPASSKCSCCH	McCormick et al., 1987
Trout (MTb)	MDP CECSKTGSCNCGGSCKCSNCACTSCKKSCCPCCPSDCSKCASGCVCKGKTC DTSCCQ	Bonham et al., 1987
Crab (MT-2)	PDP C C NDKCDCKEGECKTGCKCTSCRCPPCEQCSSGC KCANKEDCRKTCSKPCSCCP	Lerch et al., 1982
N. crassa	GDCGCSGASSCNCGSGCSCSNCGSK	Lerch, 1979
Class II		
	1 20 40 60	
Sea urchin (MTa)	MPDVKCVCCTEGKECACFGQDCCVTGECCKDGTCCGICTNAACKCANGCKCGSGCSCTEGNCAC	Nemer et al., 1985
Yeast	QNEGHECQCQCGSCKNNEQCQKSCSCPTGCNSDDKCPCGNKSEETKKSCCSGK	Winge et al., 1985
Wheat germ (E_C protein)	GCNDKCGCAVPCPGGTGCRCTSARSGAAAGEHTTCGCGEHCGGNPCACGGEGTPSGCAN[d]....	Lane et al., 1987
Cyanobacterium	TSTTLVKCACEPCLCNVDPSKAIDRNGLYYCCEACADGHTGGSKGCGHTGCNC	Olafson et al., 1988
Class III		
	1 10	
S. pombe (cadystin A[f])	eCeCeCG[e]	Kondo et al., 1984
R. canina (phytochelatin, PC_8)	eCeCeCeCeCeCeCeCG	Grill et al., 1986a
P. vulgaris (homophytochelatin, h-PC_7)	eCeCeCeCeCeCeC-β-alanine	Grill et al., 1986b

FIGURE 1: Classification of metallothioneins and amino acid sequences of representative forms (see footnote *a*). Footnotes: (a) open positions denote deletions introduced for optimal alignment of class I metallothioneins; (b) numeration refers to the sequence determined for human metallothionein 2; (c) specified subform of metallothionein; (d) partial sequence; (e) e indicates glutamic acid residue linked by γ-glutamyl bond; (f) also designated phytochelatin PC_3 (Grill et al., 1986a).

structure. Class II comprises MTs displaying none or only very distant correspondence to the mammalian forms, e.g., MTs from sea urchin, wheat, yeast, and certain prokaryotes. Class III subsumes atypical polypeptides containing γ-glutamylcysteinyl units (Robinson & Jackson, 1986).

Class I MTs display extensive genetic polymorphism (Kägi & Kojima, 1987). Mammalian tissues usually contain two major fractions, MT-1 and MT-2, differing at neutral pH by a single negative charge. In lagomorphs, ungulates, and primates there are also subforms within these fractions, separable by HPLC and specified by lower case letters, i.e., MT-1a, MT-1b, etc. The most complex polymorphism is found in the human where as much as ten isoMT genes are expressed, some of them tissue specifically.

Amino Acid Sequences and Evolution. All class I and II MTs characterized thus far are single-chain proteins. Mammalian forms contain 61 to 62 amino acid residues; chicken MT and sea urchin MTa contain 63 and 64 residues, respectively. Shorter chains are found in invertebrates and in certain fungi, the shortest one with 25 residues in *Neurospora crassa*. Class III MTs are often oligomeric structures made up of two or more polypeptide chains of variable length. Amino acid sequences are now known for over 36 class I MTs, for 4 class II MTs, and for 2 homologous sets of class III MTs [cited in Kägi and Kojima (1987)]. A representative selection is given in Figure 1. The most conspicuous feature of all forms is, besides the abundance of Cys totaling up to one-third of all residues, the frequent occurrence of Cys-X-Cys tripeptide sequences, where X is an amino acid residue other than Cys.

The hallmark of the class I MTs is the correspondence in the alignment of the Cys along the chain. In mammals all 20 Cys are invariant (Figure 2), and there is also extensive sequence homology with arthropode and certain fungal MTs (Figure 1). Also highly conserved in mammalian MTs are the basic residues Lys and Arg, which are juxtaposed to Cys and which may play an auxiliary role in the formation of the metal complexes (Pande et al., 1985). Most of the amino acid substitutions are of the conservative type. The majority of them are located in the amino-terminal half, indicating fewer evolutionary constraints on this portion of the chain. No obvious sequence relationships are discernible among members of class II MTs. Interestingly, sea urchin MT has nearly the same abundance of Cys-X-Cys and of Cys-Cys sequences as

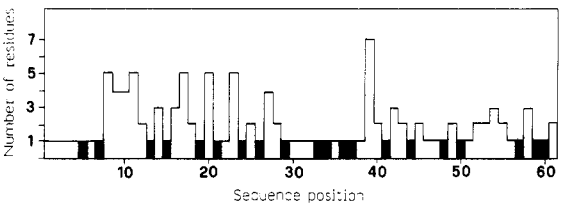

FIGURE 2: Sequence variability of metallothionein in mammals. The number of different residues found at each sequence position in 30 mammalian metallothioneins is shown. Cys are marked by filled bars [adapted from Kägi and Kojima (1987)].

mammalian MTs but with a reversed arrangement of these segments within the chain. We attribute this skewed structural correspondence to convergent evolution imposed by the requirements of metal complexation and other as yet unidentified functional constraints. The class III MTs thus far characterized are homologous, atypical oligo- and polypeptides of the general structure $(\gamma\text{Glu-Cys})_n\text{X}$, where $n = 2$–8 and X is most often glycine. The individual members of the family of $(\gamma\text{Glu-Cys})_n\text{Gly}$ are homologues of glutathione and have been designated as phytochelatins (PC_n). Seven phytochelatins (PC_2–PC_8) have been identified in tissues and cell cultures of a number of higher plants and in *Schizosaccharomyces pombe* exposed to Cd salts (Grill et al., 1986a). Some plants contain homologues of PC_n in which the C-terminal glycine is replaced by β-alanine (Grill et al., 1986b) or serine.

From amino acid sequence comparison of the class I MTs of a marine arthropod and of chicken with those of mammals, a unit evolutionary period of 14 and 16.5 million years, respectively, has been calculated (Hunziker & Kägi, 1985; McCormick et al., 1988). Thus, class I MTs are rather slowly evolving proteins with a rate intermediate between those of cytochrome *c* and hemoglobin. The occurrence of closely related isoMTs in distant lines of descent suggests various independent gene duplication events.

Metal-Binding Sites. The abundance of Cys and their conspicuous arrangement in chelating Cys-Cys, Cys-X-Cys, and Cys-X-Y-Cys, where X and Y are residues other than Cys, predispose MT toward the binding of "soft" metal ions. In mammalian MTs, these ligands collectively accommodate seven bivalent metal ions, and there is no indication that tissues and cells contain appreciable amounts of metal-deficient forms.

However, there is very often a striking variability in metal composition. Thus, preparations of native mammalian MTs judged to be pure by protein–chemical criteria can be heterogeneous with respect to the proportions of Zn, Cd, Cu, and minor metallic constituents. To a certain extent, the metal ion composition is determined by the supply of the metals to the organisms [cited in Nordberg and Kojima (1979)]. In most mammals not exposed to experimental pretreatment, Zn tends to prevail over Cd and Cu. In human liver MTs, Zn is often the sole metallic component.

Zn and Cd are readily released from the protein moiety by acidification. From the resulting apoMT and appropriate amounts of metal salts, homogeneously substituted derivatives, each containing 7 equiv of Zn, Cd, Hg, Pb, Bi, Sn, Co, Ni, Bi, or TcO, have been prepared and characterized [cited in Kägi and Kojima (1987)]. Derivatives with higher metal-to-protein stoichiometries are observed with univalent d^{10} ions, such as Cu(I), Ag(I), and Au(I). The affinity of the metal ions for the binding sites of mammalian MTs follows the order of thiolate model complexes, i.e., Zn(II) < Pb(II) < Cd(II) < Cu(I), Ag(I), Hg(II), Bi(III). At neutral pH, for the first three metal ions average apparent stability constants were calculated to be approximately $K'_{Zn} = 2 \times 10^{-12}$ M^{-1}, $K'_{Pb} = 4 \times 10^{-14}$ M^{-1}, and $K'_{Cd} = 2 \times 10^{-16}$ M^{-1} [cited in Kägi and Kojima (1987)]. The 10 000-fold higher affinity for Cd(II) over Zn(II) is thought to be the reason for the tendency of the environmentally much less abundant Cd to be accumulated in MT.

In native mammalian MTs all 20 Cys are deprotonated and participate in metal binding. The geometric details of metal coordination have been explored in a variety of spectroscopic studies of native and reconstituted forms of mammalian MTs (Vašák & Kägi, 1983). They showed that the complexes are chemically and structurally uniform with binding of each bivalent metal to four thiolate ligands arranged in tetrahedral-like symmetry. This MS_4 coordination geometry was first inferred from the homology between the lowest energy bands in the far-UV absorption spectra of Zn-, Cd-, and Hg-containing forms of MT and those of tetrahedral halide complexes of the same metal ions, a feature that also allowed for the assignment of the first absorption bands to ligand–metal charge-transfer transitions (Vašák et al., 1981a). Tetrahedral tetrathiolate coordination was also suggested by the chemical shifts of ^{113}Cd NMR signals of MT and from interatomic Cd–S and Zn–S distances determined by EXAFS measurements of the Cd- and/or Zn-containing forms [cited in Kägi and Kojima (1987)].

More direct evidence for a tetrahedral arrangement came from the measurement of the perturbation of the angular correlation (PAC) of γ-ray emission of the metastable nuclide ^{111m}Cd incorporated into MT (Vašák & Bauer, 1982) and from spectroscopic and magnetooptical studies of forms of MT reconstituted with Co(II), Ni(II) (Vašák et al., 1981b), and Fe(II) (Good & Vašák, 1986). Both Co_7- and Fe_7-MTs show the spin-allowed $\nu_2[^4A_2 \rightarrow {}^4T_1(F)]$ and $\nu_2[^5E \rightarrow {}^5T_2]$ transitions, respectively, diagnostic of tetrahedral symmetry. Independent confirmation that all seven metal ions are tetrahedrally coordinated to the sulfur of the Cys side chains has also come from X-ray crystallographic analysis (Furey et al., 1986). No information is as yet available on the coordination geometry of class III MTs. Some Cd-containing phytochelatins contain acid-labile inorganic sulfide (Hayashi et al., 1986).

Metal–Thiolate Clusters. The cardinal feature of all classes of MT is the organization of the metal complexes in metal–thiolate clusters. This arrangement, which allows sharing of some of the thiolate ligands by adjacent metal ions, reconciles the tetrahedral tetrathiolate coordination with the measured stoichiometry of nearly three thiolate ligands per bivalent metal ion bound. In mammalian MTs the ratio of 20 Cys to 7 metal ions requires that 8 Cys serve as doubly coordinated bridging thiolate ligands and 12 as singly coordinated terminal thiolate ligands.

The most direct evidence for the linkage of metal ions by thiolate bridges was obtained in the ^{113}Cd NMR studies of ^{113}Cd-enriched rabbit liver MT by Otvos and Armitage (1980), who observed splitting of the ^{113}Cd resonances as a result of ^{113}Cd–^{113}Cd scalar coupling via thiolate ligands. Analogous effects involving superexchange interactions of unpaired electrons were observed by ESR measurements of MT in which diamagnetic Cd(II) and Zn(II) were replaced by paramagnetic Co(II) (Vašák & Kägi, 1981). This effect, which was also observed in crab and *Neurospora crassa* MT (Beltramini et al., 1984) as well as in crystallographically defined model clusters, composed of four tetrahedral Co(II) benzene–thiolate complexes (Dance, 1979), is associated with a reduced magnetic moment attributed to partial spin canceling. Additional evidence for the clustered arrangement of the metal ions was obtained from EXAFS measurements of mammalian Zn_7-MT (Abrahams et al., 1986) and *N. crassa* Cu_6-MT (Smith et al., 1986), documenting backscattering from neighboring metal ions. It is also evidenced by a variety of electronic spectroscopic features. Thus, the ligand–metal charge-transfer absorption bands of the Co_7, Fe_7, Cd_7, and Hg_7 derivatives of MT are red-shifted with respect to the position of these bands in mononuclear tetrahedral tetrathiolate complexes formed at lower metal-to-ligand ratios [cited in Kägi and Kojima (1987)]. Corresponding shifts are observed in the Faraday *A*-term MCD signals associated with the same transition. The most conspicuous spectroscopic manifestations of the clusters are the multiphasic ORD and CD profiles observed in all classes of MT. The effect originates from excitonic coupling of transitions located at the bridging thiolate ligands (Willner et al., 1987).

In mammalian and crab MTs the metal ions are partitioned into two topologically separate metal–thiolate clusters. This was shown by homonuclear decoupling studies, which allowed the allocation of the seven ^{113}Cd NMR resonances of $^{113}Cd_7$-MT from rabbit liver to two linkage groups, one with 4 Cd and 11 Cys, i.e., Cd_4Cys_{11} or cluster A, and the other with 3 Cd and 9 Cys, i.e., Cd_3Cys_9 or cluster B (Otvos & Armitage, 1980). In cluster A, the four metal ions coupled through five bridging thiolate ligands were pictured to form a bicyclo[3.3.1]nonane-like structure made up of two six-membered rings positioned at virtually right angles to each other. It is inferred that in cluster B three metal ions and three bridging thiolate cysteines form a six-membered ring. This partitioning, into two completely separate clusters, was independently verified by limited enzymic proteolysis, which resulted in a bisection of the protein into a carboxyl- and an amino-terminal portion, designated as α- and β-domain and containing the Cys ligands of cluster A and cluster B, respectively (Winge & Miklossy, 1982). A ^{113}Cd NMR decoupling analysis of ^{113}Cd-enriched MT from the hepatopancreas of the marine crab *Scylla serrata* allowed the deduction of two similarly structured Cd_3Cys_9 clusters (Otvos et al., 1982).

Spatial Structure. Models for the spatial structure of mammalian MT and the organization of the metal–thiolate clusters have recently been derived both from 2D NMR

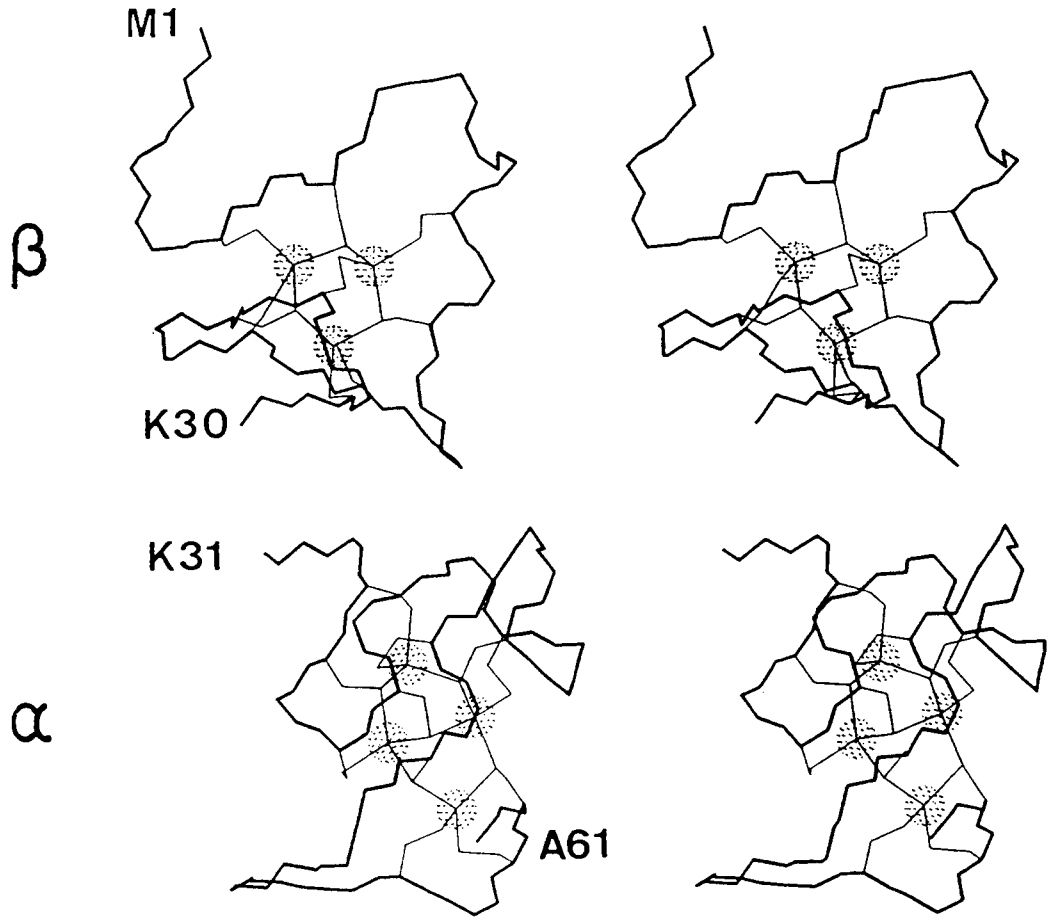

FIGURE 3: Two-dimensional NMR solution structure of β-domain (top) and α-domain (bottom) of rat liver Cd_7-MT-2. Stereoview of polypeptide backbone (thick line), Cys side chains (thin lines), and metal positions (dotted spheres of radius 0.9 Å). The figures are prepared from the coordinates of the best RMS structures of Schultze et al. (1988) (courtesy of P. Schultze).

spectroscopic measurements in aqueous solution (Braun et al., 1986; Arseniev et al., 1988; Schultze et al., 1988) and from X-ray diffraction data obtained on crystals (Furey et al., 1986). A stereoview of the polypeptide backbone, the Cys side chains, and the metal positions of the two domains of ^{113}Cd-substituted MT-2 from rat liver as determined by homonuclear and heteronuclear two-dimensional NMR techniques, is shown in Figure 3. The uniformly sized and nearly spherical α- and β-domains have a diameter of 15–20 Å and contain in their center the respective metal–thiolate clusters as "mineral cores" around each of which the polypeptide chain is wrapped, forming two large helical turns. In the carboxyl-terminal α-domain the spiral of the peptide fold is left-handed and in the amino-terminal β-domain it is right-handed. The domains are connected by a hinge region consisting of the conserved Lys-Lys-Ser segment in the middle of the polypeptide chain. However, owing to the paucity of clearly recognizable interdomain contacts, the mutual orientation of the domains has not as yet been defined. This failure probably reflects a certain flexibility about the hinge region.

The keystone of the 2D NMR solution structure analysis of MT is the direct and unambiguous identification of all 28 Cys–Cd bonds by heteronuclear 2D ^{1}H ^{113}Cd NMR correlation measurements (Frey et al., 1985). The connectivities that also localize all eight bridging Cys of the two clusters of rat liver MT-2 are displayed in Figure 4. Exactly the same structural organization of the clusters with the same Cys–Cd connectivities and with almost superimposable polypeptide folds was found for rabbit liver MT-2a (Arseniev et al., 1988). This agreement of two mammalian MTs, differing in nearly one-fourth of all non-cysteine residues, documents that cluster

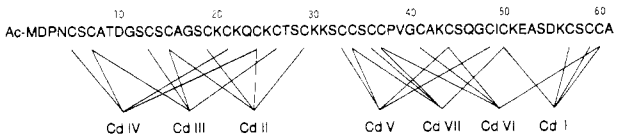

FIGURE 4: Cadmium–cysteine connectivities of rat liver metallothionein 2 as established by two-dimensional ^{1}H ^{113}Cd NMR spectroscopy [adapted from Vašák et al. (1987)]. Roman numerals refer to the ^{113}Cd resonances assigned by Otvos and Armitage (1980).

formation and polypeptide folding are determined by the conserved arrangement of the 20 Cys in the chain. The anchoring of all 20 Cys side chains by their coordination bonds to metal ions in the interior of the domains severely restricts the polypeptide chain conformation between the Cys. Thus, rat and rabbit MTs display numerous unusual secondary structure elements including two 3/10 helical segments and several "half-turns", a hydrogen-bonded structure motif thus far recognized only in the MTs (Wagner et al., 1986; Arseniev et al., 1988; Schultze et al., 1988). The abundance of turn structures was also noted in the Raman spectrum of rabbit liver MT-1 (Pande et al., 1986).

The model derived from X-ray crystallographic analysis of native rat liver MT-2 resembles the 2D NMR solution structure in its overall organization including the steric organization of the clusters, the general disposition of the polypeptide chain, and the handedness of the folds (Furey et al., 1986). However, there are very extensive discrepancies in the detailed spatial arrangements (Vašák et al., 1987; Schultze et al., 1988). Thus, there are large differences in the positioning of segments of the chain and consequently only 25% of all Cys agree, with respect to metal coordination, between

Table I: Factors That Induce Metallothionein Synthesis in Cultured Cells or in Vivo[a]

metal ions: Cd, Zn, Cu, Hg, Au, Ag, Co, Ni, Bi	streptozotocin
	2-propanol
	ethanol
glucocorticoids	ethionine
progesterone	alkylating agents
estrogen	chloroform
glucagon	carbon tetrachloride
catecholamines	
interleukin I	starvation
interferon	infection
	inflammation
butyrate	laparatomy
retinoate	physical stress
phorbol esters	X-irradiation
endotoxin	high O_2 tension
carrageenan	
dextran	

[a] For citations, see Palmiter (1987) and Bremner (1987).

the two structures (Wagner et al., 1987). The cause of these differences remains to be clarified.

Dynamic Aspects. The unique feature of the polypeptide fold of mammalian MT is its stabilization by a total of 42 intramolecular Cys-metal-Cys cross-links. With 24 of these connections in the α-domain and only 18 in the β-domain, the conformational stability and the collective affinity for the metal are expectedly lower in the latter. Thus, the Cys side chains of the β-domain are more accessible to alkylating agents, and cluster B has a greater tendency to lose metal (Bernhard et al., 1986). This distinct difference in affinity for metals is also reflected by the sequence in which the clusters are built up when bivalent post-transition-metal ions are added to the apoprotein (Nielson & Winge, 1983).

Complexes of group IIB metal ions with thiolate ligands are highly motile with exchange rates increasing from Zn(II) to Cd(II) to Hg(II) (Carson & Dean, 1982). Thus, the Cys-metal-Cys cross-links in MT, while thermodynamically stable (see above), are expected to be kinetically labile, allowing for fluxional breaking and re-forming of the coordination bonds. This surmised nonrigidity of the clusters is supported experimentally by a number of spectroscopic features interpretable in terms of dynamic fluctuations (Vašák, 1986). The most direct evidence is the rapid intramolecular exchange of ^{113}Cd among binding sites in MT monitored by saturation-transfer NMR spectroscopy (Otvos et al., 1987). The exchange that is particularly fast in cluster B is also thought to underlie the remarkably facile redistribution of metal ions observed between $^{113}Cd_7$-MT and Zn_7-MT (Nettesheim et al., 1985).

Molecular Biology. A most remarkable biological feature of all MTs is their inducibility. Early observations established that tissue contents of MT are increased by the administration of Cd salts (Piscator, 1964). Other factors stimulating MT biosynthesis in cultured cells or in vivo include several other metals, hormones, a still growing number of cytotoxic agents, and various pathophysiological conditions associated with physical or chemical stress (Table I). The induction occurs at the level of transcription initiation, leading to maximum MT concentrations within 1–2 days after exposure to an inducer [cited in Nordberg and Kojima (1979)]. Half-lives of MT are of the order of 1–4 days, depending on the type of metal bound (Cousins, 1983). In rodent cell lines the activation of both isoMT genes is coordinate (Griffith, 1985). In human cells, expression of some of the isoMT genes appears to be regulated differentially by Cd, Zn, and glucocorticoids (Richards et al., 1984), and there are indications for tissue-specific expression (Schmidt & Hamer, 1986; Varshney et al., 1986).

A number of studies have led to the identification of various DNA segments serving as promoter sites in the 5′ region of various MT genes in the induction by metal ions and hormones [cited in Palmiter (1987)]. In the mouse MT-1 gene, the functional metal-responsive promoter is composed of a set of four closely related metal-regulatory elements, each made up of eight nucleotides and localized near the TATA box (Stuart et al., 1985). It is thought to be recognized by one or more MT gene-binding proteins acting as positive transcription factors when activated by the appropriate metal ion (Seguin & Hamer, 1987). Because the metal responsive MT promoter was shown to function in quite different cell types and independent of species and since it is susceptible to regulation by the metal supply, it has found an important technical application in the so-called MT fusion genes [cited in Palmiter (1987)]. In these constructs the promoter is linked to a structural gene of interest, thereby allowing modulation of expression of its gene product in cultured cells by metals. Genes employing the Cu-sensitive promoter of yeast Cu-MT are expected to have applications in the biotechnological and the biopharmaceutical synthesis of proteins in yeast (Butt & Ecker, 1987). Fusion genes containing the mouse MT-1 promoter have also been employed in transgenic experiments, allowing regulation of the introduced gene by metal supplementation (Palmiter & Brinster, 1985).

Functional Aspects. More than 3 decades after the discovery of MT, its functional significance remains a topic of discussion (Karin, 1985; Bremner, 1987). The conservation of the structure, the ubiquitous occurrence, and the programmed synthesis in regeneration and development of MT are strong arguments for its playing a crucial role in some fundamental metal-related cell-biological processes. The main hypotheses thus far considered are that (1) MT serves as a rather unspecific metal-buffering ligand to either sequester or dispense metal ions or that (2) it has a specialized function in normal cellular metabolism or development. Pleiotropically, it may well serve a number of different biological purposes.

That MT is the cellular component responsible for much of the intracellular sequestration of Cd bringing about the long biological half-life of this nonessential element is unquestioned (Webb, 1987a). There is also good evidence that owing to its inducibility MT provides animals and cells with a mechanism to attenuate at least temporarily the toxicity of Cd [cited in Nordberg and Kojima (1979)]. In cultured mammalian cell lines stable resistance to Cd can be brought about by massive amplification of the MT genes (Beach & Palmiter, 1981). However, the production of excessive amounts of Cd-containing MT has been been suggested as a causative factor in bringing about kidney damage in chronic Cd poisoning (Nordberg & Nordberg, 1987), thus casting doubt on the biological importance of MT synthesis as a specific and effective defense mechanism in animals against environmental Cd. Whether or not binding of Cd by class III MTs in plant cells affords effective protection from this metal remains to be established (Robinson & Jackson, 1986).

MTs have also been implicated in the sequestration of nonessential metals, such as Hg, Pb, Bi, Ag, Au, and Pt [cited in Webb (1987a)]. Such effects have been claimed to be responsible for the development of resistance toward Au- and Pt-containing drugs in cultured cells and for the selective protection of some tissues from such agents in animals following preinduction of MT (Naganuma et al., 1987; Monia et al., 1987).

The preponderance of Zn in most preparations of MT and the responsiveness of MT-bound Zn and Cu to the dietary

supplies of these essential nutrients are arguments for a role in their metabolism and have led to a large number of studies [cited in Bremner (1987)]. As a homeostatic mediator MT could donate metal ions in the biosynthesis of Zn- and Cu-containing metalloenzymes and metalloproteins. The emergence of Cu_6-MT in *N. crassa* prior to the formation of the Cu-containing enzymes tyrosinase and laccase would be in concert with such a role (Huber & Lerch, 1987) as are in vitro experiments which have demonstrated that Cu and Zn can be transferred from MT to the apo forms of a number of Cu and Zn proteins, respectively (Beltramini & Lerch, 1982; Brady, 1982). Conversely, when Cu and Zn accumulate intracellularly, the reactive ions can be sequestered in a chemically innocuous form by binding to newly synthesized apoMT. This mechanism is believed to account for the accumulation of large amounts of Cu-MT in tissues and cells of organisms affected with inherited disorders of Cu metabolism (Sternlieb, 1987; Packman et al., 1987). Protection from the effects of excessive ionic Cu by sequestration is thus far the only documented benefit of MT induction in yeast (Thiele et al., 1986; Ecker et al., 1986), in *N. crassa* (Lerch & Beltramini, 1983), and in copper-resistant forms of *Agrostis gigantea* (Rauser & Curvetto, 1980).

A biological role, probably unrelated to a detoxification function for metals, is suggested by the fact that in certain tissues and cell types MT is induced by many forms of chemical and physical stress (Table I). These effects, which are most prominent in liver and mediated in part by hormones, resemble an acute phase response (Bremner, 1987). In some cases, such as in the exposure to electrophilic agents, i.e., O_2, free radicals, and alkylating agents, the increased supply of MT could provide the "neutralizing" nucleophilic equivalents. However, in most other instances it is unclear as yet in what way the organism benefits from increased MT biosynthesis.

An intriguing thought is that MTs may have a metallooregulatory function in cellular repair processes, growth, and differentiation. This was first suggested by the parallelism of enhanced DNA synthesis with increased Zn-MT formation observed in the liver of rats recovering from partial hepatectomy (Ohtake et al., 1978) and is supported by the programmed regulation of MT mRNA levels and of MT in the course of embryogenesis (Nemer et al., 1984) and in different stages of fetal (Andrews et al., 1984) and perinatal (Bakka et al., 1981; Panamangalore et al., 1983) development. There are also reports on a differential activation of MT isoforms in these processes [cited in Webb (1987b) and Wilkinson and Nemer (1987)]. In view of the known effects of Zn on embryogenesis, its participation in a number of DNA and RNA polymerases (Wu & Wu, 1987), and its serving as a structural modulator of the Zn finger domains in several DNA-binding proteins, it is tempting to hypothesize that Zn-MT plays a part in the storage, transmission, and expression of genetic information. The analogies in the Zn-binding sequence motifs between DNA-binding proteins (Klug & Rhodes, 1987) and MT could imply a similar metal-regulated interaction of the latter with nucleic acids.

References

Abrahams, I. L., Bremner, I., Diakun, G. P., Garner, C. D., Hasnain, S. S., Ross, I., & Vašák, M. (1986) *Biochem. J. 236*, 585–589.

Andrews, G. K., Adamson, E. D., & Gedamu, L. (1984) *Dev. Biol. 103*, 294–303.

Arseniev, A., Schultze, P., Wörgötter, E., Braun, W., Wagner, G., Vašák, M., Kägi, J. H. R., & Wüthrich, K. (1988) *J. Mol. Biol. 201*, 637–657.

Bakka, A., Samarawickrama, G. P., & Webb, M. (1981) *Chem.-Biol. Interact. 34*, 161–171.

Beach, L. R., & Palmiter, R. D. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2110–2114.

Beltramini, M., & Lerch, K. (1982) *FEBS Lett. 142*, 219–222.

Beltramini, M., Lerch, K., & Vašák, M. (1984) *Biochemistry 23*, 3422–3427.

Bernhard, W. R. Vašák, M., & Kägi, J. H. R. (1986) *Biochemistry 25*, 1975–1980.

Bonham, K., Zafarullah, M., & Gedamu, L. (1987) *DNA 6*, 519–528.

Brady, F. O. (1982) *Trends Biochem. Sci. (Pers. Ed.) 7*, 143–145.

Braun, W., Wagner, G., Wörgötter, E., Vašák, M., Kägi, J. H. R., & Wüthrich, K. (1986) *J. Mol. Biol. 187*, 125–129.

Bremner, I. (1987) *Experientia, Suppl. 52*, 81–107.

Butt, T. R., & Ecker, D. J. (1987) *Microbiol. Rev. 51*, 351–364.

Carson, G. K., & Dean, P. A. W. (1982) *Inorg. Chim. Acta 66*, 157–161.

Cousins, R. J. (1983) *J. Inherited Metab. Dis. 6 (Suppl. 1)*, 15–21.

Dance, I. G. (1979) *J. Am. Chem. Soc. 101*, 6264–6273.

Ecker, D. J., Butt, T. R., Sternberg, E. J., Neeper, M. P., Debouck, C., Gorman, J. A., & Crooke, S. T. (1986) *J. Biol. Chem. 261*, 16895–16900.

Fowler, B. A., Hildebrand, C. E., Kojima, Y., & Webb, M. (1987) *Experientia, Suppl. 52*, 19–22.

Frey, M. H., Wagner, G., Vašák, M., Sørensen, O. W., Neuhaus, D., Wörgötter, E., Kägi, J. H. R., Ernst, R. R., & Wüthrick, K. (1985) *J. Am. Chem. Soc. 107*, 6847–6851.

Furey, W. F., Robbins, A. H., Clancy, L. L., Winge, D. R., Wang, B. C., & Stout, C. D. (1986) *Science (Washington, D.C.) 231*, 704–710.

Good, M., & Vašák, M. (1986) *Biochemistry 25*, 8353–8356.

Griffith, J. K. (1985) *Mol. Cell. Biol. 5*, 3525–3531.

Grill, E., Winnacker, E.-L., & Zenk, M. H. (1986a) *FEBS Lett. 197*, 115–120.

Grill, E., Gekeler, W., Winnacker, E.-L., & Zenk, M. H. (1986b) *FEBS Lett. 205*, 47–50.

Hamer, D. H. (1986) *Annu. Rev. Biochem. 55*, 913–951.

Hayashi, Y., Nakagawa, C. W., & Murasugi, A. (1986) *Environ. Health Perspect. 65*, 13–19.

Huber, M., & Lerch, K. (1987) *FEBS Lett. 219*, 335–338.

Hunziker, P. E., & Kägi, J. H. R. (1985) in *Metalloproteins* (Harrison, P. M., Ed.) Part 2, pp 149–181, Macmillan, London.

Johnson, G. F., Morell, A. G., Stockert, R. J., & Sternlieb, I. (1981) *Hepatology (Baltimore) 1*, 243–248.

Kägi, J. H. R., & Vallee, B. L. (1960) *J. Biol. Chem. 235*, 3460–3465.

Kägi, J. H. R., & Vallee, B. L. (1961) *J. Biol. Chem. 236*, 2435–2442.

Kägi, J. H. R., & Kojima, Y. (1987) *Experientia, Suppl. 52*, 25–61.

Karin, M. (1985) *Cell (Cambridge, Mass.) 41*, 9–10.

Kissling, M. M., & Kägi, J. H. R. (1977) *FEBS Lett. 82*, 247–250.

Klug, A., & Rhodes, D. (1987) *Trends Biochem. Sci. (Pers. Ed.) 12*, 464–469.

Kondo, N., Imai, K., Isobe, M., Goto, T., Murasugi, A., Wada-Nakagawa, C., & Hayashi, Y. (1984) *Tetrahedron Lett. 25*, 3869–3872.

Lane, B., Kajioka, R., & Kennedy, T. (1987) *Biochem. Cell Biol. 65*, 1001–1005.

Lerch, K. (1979) *Experientia, Suppl. 34*, 173–179.
Lerch, K., & Beltramini, M. (1983) *Chem. Scr. 21*, 109–115.
Lerch, K., Ammer, D., & Olafson, R. W. (1982) *J. Biol. Chem. 257*, 2420–2426.
Margoshes, M., & Vallee, B. L. (1957) *J. Am. Chem. Soc. 79*, 4813–4814.
McCormick, C. C., Fullmer, C. S., & Garvey, J. S. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 309–313.
Monia, B. P., Butt, T. R., Mirabelli, C. K., Ecker, D. J., Sternberg, E., & Crooke, S. T. (1987) *Mol. Pharmacol. 31*, 21–26.
Naganuma, A., Satoh, M., & Imura, N. (1987) *Cancer Res. 47*, 983–987.
Nartey, N. O., Banerjee, D., & Cherian, M. G. (1987) *Pathology 19*, 233–238.
Nemer, M., Travaglini, E. C., Rondinell, E., & D'Alonzo, J. (1984) *Dev. Biol. 102*, 471–482.
Nemer, M., Wilkinson, D. G., Travaglini, E. C., Sternberg, E. J., & Butt, T. R. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4992–4994.
Nettesheim, D. G., Engeseth, H. R., & Otvos, J. D. (1985) *Biochemistry 24*, 6744–6751.
Nielson, K. B., & Winge, D. R. (1983) *J. Biol. Chem. 258*, 13063–13069.
Nordberg, M., & Kojima, Y. (1979) *Experientia, Suppl. 34*, 41–124.
Nordberg, M., & Nordberg, G. F. (1987) *Experientia, Suppl. 52*, 669–675.
Ohtake, H., Hasegawa, K., & Koga, M. (1978) *Biochem. J. 174*, 999–1005.
Olafson, R. W., McCubbin, W. D., & Kay, C. M. (1988) *Biochem. J. 251*, 691–699.
Otvos, J. D., & Armitage, I. M. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 7094–7098.
Otvos, J. D., Olafson, R. W., & Armitage, I. M. (1982) *J. Biol. Chem. 257*, 2427–2431.
Otvos, J. D., Engeseth, H. R., Nettesheim, D. G., & Hilt, C. R. (1987) *Experientia, Suppl. 52*, 171–178.
Packman, S., Palmiter, R. D., Karin, M., & O'Toole, C. (1987) *J. Clin. Invest. 79*, 1338–1342.
Palmiter, R. D. (1987) *Experientia, Suppl. 52*, 63–80.
Palmiter, R. D., & Brinster, R. L. (1985) *Cell (Cambridge, Mass.) 41*, 343–345.
Pande, J., Vašák, M., & Kägi, J. H. R. (1985) *Biochemistry 24*, 6717–6722.
Pande, J., Pande, C., Gilg, D., Vašák, M., Callender, R., & Kägi, J. H. R. (1986) *Biochemistry 25*, 5526–5532.
Panemangalore, M., Banerjee, D., Onosaka, S., & Cherian, M. G. (1983) *Dev. Biol. 97*, 95–102.
Piscator, M. (1964) *Nord. Hyg. Tidskr. 45*, 76–82.
Pulido, P., Kägi, J. H. R., & Vallee, B. L. (1966) *Biochemistry 5*, 1768–1777.
Rauser, W. E., & Curvetto, N. R. (1980) *Nature (London) 287*, 563–564.
Richards, R. I., Heguy, A., & Karin, M. (1984) *Cell (Cambrige, Mass.) 37*, 263–272.
Robinson, N. J., & Jackson, P. J. (1986) *Physiol. Plant. 67*, 499–506.
Schmidt, C. J., & Hamer, D. H. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3346–3350.
Schultze, P., Wörgötter, E., Braun, W., Wagner, G., Vašák, M., Kägi, J. H. R., & Wüthrich, K. (1988) *J. Mol. Biol.* (in press).
Seguin, C., & Hamer, D. H. (1987) *Science (Washington, D.C.) 235*, 1383–1387.
Smith, T. A., Lerch, K., & Hodgson, K. O. (1986) *Inorg. Chem. 25*, 4677–4680.
Sternlieb, I. (1987) *Experientia, Suppl. 52*, 647–653.
Stuart, G. W., Searle, P. F., & Palmiter, R. D. (1985) *Nature (London) 317*, 828–831.
Thiele, D. J., Walling, M. J., & Hamer, D. H. (1986) *Science (Washington, D.C.) 231*, 854–856.
Vallee, B. L. (1960) *Proc. Int. Congr. Biochem., 4th, 1958 8*, 138–166.
Varshney, U., Jahroudi, N., Foster, R., & Gedamu, L. (1986) *Mol. Cell. Biol. 6*, 26–37.
Vašák, M. (1986) *Environ. Health Perspect. 65*, 193–197.
Vašák, M., & Kägi, J. H. R. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 6709–6713.
Vašák, M., & Bauer, R. (1982) *J. Am. Chem. Soc. 104*, 3236–3238.
Vašák, M., & Kägi, J. H. R. (1983) *Met. Ions Biol. Syst. 15*, 213–273.
Vašák, M., Kägi, J. H. R., & Hill, H. A. O. (1981a) *Biochemistry 20*, 2852–2856.
Vašák, M., Kägi, J. H. R., Holmquist, B., & Vallee, B. L. (1981b) *Biochemistry 20*, 6659–6664.
Vašák, M., Wörgötter, E., Wagner, G., Kägi, J. H. R., & Wüthrich, K. (1987) *J. Mol. Biol. 196*, 711–719.
Wagner, G., Neuhaus, D., Wörgötter, E., Vašák, M., Kägi, J. H. R., & Wüthrich, K. (1986) *J. Mol. Biol. 187*, 131–135.
Wagner, G., Frey, M. H., Neuhaus, D., Wörgötter, E., Braun, W., Vašák, M., Kägi, J. H. R., & Wüthrich, K. (1987) *Experientia, Suppl. 52*, 149–157.
Webb, M. (1987a) *Experientia, Suppl. 52*, 109–134.
Webb, M. (1987b) *Experientia, Suppl. 52*, 483–498.
Wilkinson, D. G., & Nemer, M. (1987) *Mol. Cell. Biol. 7*, 48–58.
Willner, H., Vašák, M., & Kägi, J. H. R. (1987) *Biochemistry 26*, 6287–6292.
Winge, D. R., & Miklossy, K.-A. (1982) *J. Biol. Chem. 257*, 3471–3476.
Winge, D. R., Nielson, K. B., Gray, W. R., & Hamer, D. H. (1985) *J. Biol. Chem. 260*, 14464–14470.
Wu, F. Y.-H., & Wu, C.-W. (1987) *Annu. Rev. Nutr. 7*, 251–272.