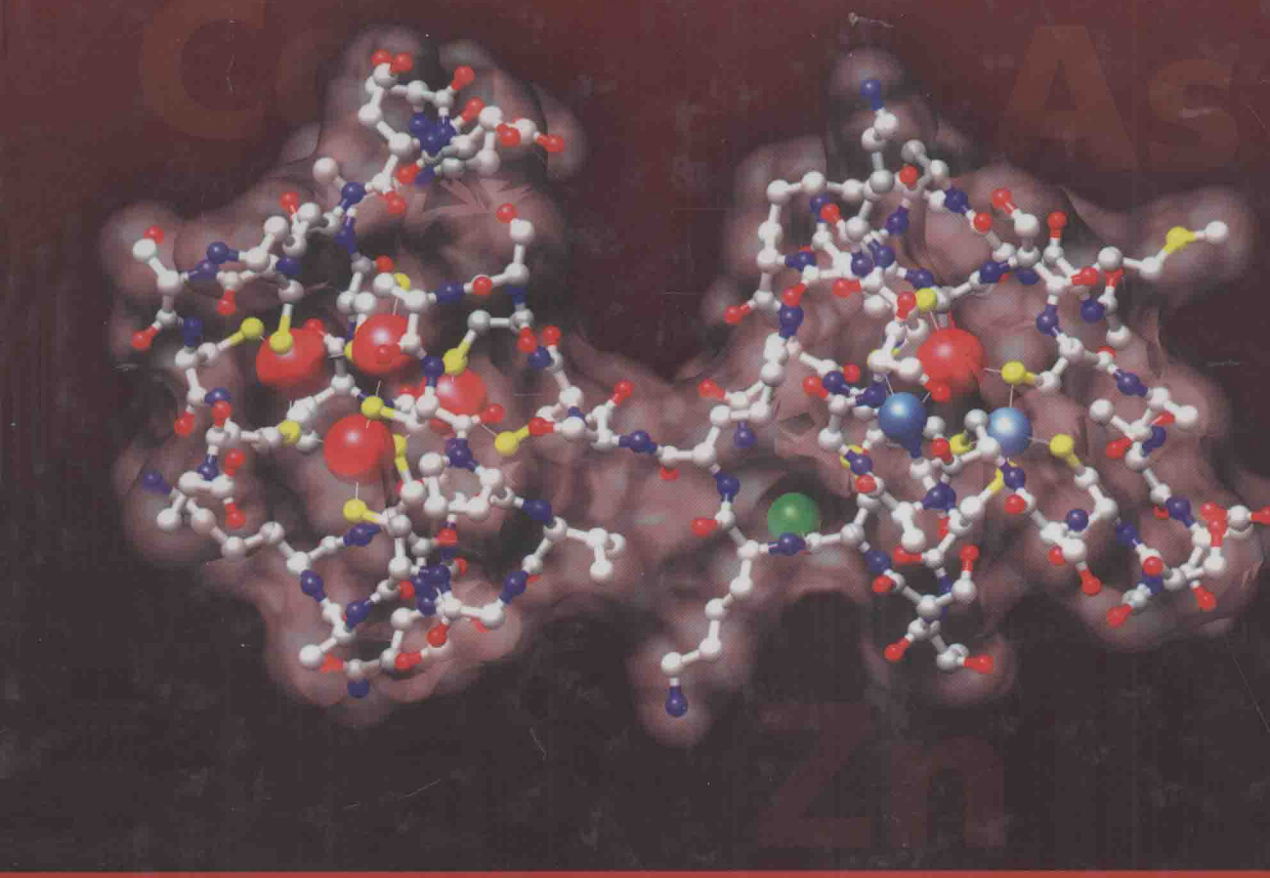
Cellular and Molecular Biology of Metals



Edited by
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CRC Press
Taylor & Francis Group

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

The cover displays a space-filled rendering of rat Metallothionein II as generated from the Brookhaven Protein Databank file 4MT2 (submitted by A.H. Robbins and C.D. Stout). The image was rendered by Rudolfs K. Zalups, Ph.D. using the molecular drawing program Chimera, which is supplied by University of California at San Francisco.

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number: 978-1-4200-5997-7 (Hardback)

Library of Congress Cataloging-in-Publication Data

Cellular and molecular biology of metals / editors, Rudolfs K. Zalups and D. James Koropatnick.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-4200-5997-7 (hardcover : alk. paper)
1. Metals--Physiological effect. 2. Metals--Metabolism. 3. Metals--Toxicology. 4. Cytochemistry. I. Zalups, Rudolfs K. II. Koropatnick, Donald James, 1952- III. Title.
[DNLM: 1. Metals--metabolism. 2. Metals--toxicity. 3. Biological Transport--physiology. 4. Cell Physiological Phenomena--drug effects. 5. Molecular Biology--methods. QV 275 C393 2010]

QP532.C45 2010
615.9'253--dc22 2009053540

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and the CRC Press Web site at
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Preface

Despite all the progress being made in the fields of molecular and cellular biology, the role and effects of metal ions on cellular homeostasis in the various organs of mammals are only beginning to be truly defined. Working with divalent and trivalent metals and metals with higher valency in biological systems can be particularly challenging because of the complex and, under certain conditions, transient bonding interactions that metal ions can undergo. It is particularly challenging to follow metal ions in their complex biological journey from the environment in tissues and cells. That journey commonly involves association of metals with extracellular ligands that are either specific to particular metal species or promiscuous in their associations with metals, then entry into the cytosolic compartment of target cells. Cell entry requires metals to traverse the cellular plasma membrane, often through the interaction of metals, their ligands, or both, with transporter molecules or by mechanisms independent of transporters. Intracellular metal ions then associate with intracellular molecules in specific compartments to signal their presence and trigger cellular responses to that presence, and to carry out physiological functions as essential components of cellular enzymes and structural molecules.

Moreover, without the availability of radioactive forms of certain metal ions, accurate measurement of metal content within target cells and their subcellular compartments and organelles exceeds the sensitivity, accuracy, and reproducibility of current quantitative and qualitative analytical methods to measure these metallic species. With the continued decrease in commercially available isotopes of various metals, new challenges are being imposed on the next generation of molecular and cellular biologists. We rely on them for new methods and experimental strategies to discover how mammalian cells detect, take up, use, and excrete metals to maximize their extraordinarily valuable reductive and oxidative capacity for cellular function while minimizing their capacity for harm—and to exploit that knowledge for therapeutic benefit and to avoid metal-induced damage.

Our rationale for this volume stems from the ever-shifting sands of opportunity to compile a written summary of the state of knowledge in metal metabolism and homeostasis in target cells. We have compiled the current perspectives of experts in the areas of transport and handling, metabolism, and transcriptional regulatory activity of a number of metal ions of high current interest in the scientific literature.

Unlike our previous volume (*Molecular Biology and Toxicology of Metals*, published in 2000 by Taylor & Francis), which focused on the toxicology effects of a number of metals, the present volume concentrates primarily on physiological mechanisms underlying metal ion handling with respect to homeostasis, enzyme activity, transcriptional regulation, and other events designed to avoid toxicity and enhance cellular function. In view of the long life (indeed, the immortality) of metal ions, their capacity to both nurture and damage living systems, and their exceptional value as molecular redox tools in the hands of cellular molecules, the subject continues to both fascinate and generate new knowledge with the potential to reframe our understanding of cellular function.

James Koropatnick
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Editors

Rudolfs K. Zalups attended the State University of New York (SUNY) College at Brockport as an undergraduate where he received a B.S. in mathematics and electronic music. He later received a M.S. in zoology from SUNY Brockport and a doctorate in human anatomy and cell biology at the University of Western Ontario in London, Ontario, Canada. He continued his training as a fellow and instructor at the Mayo Clinic, Yale University School of Medicine, University of Maryland School of Medicine and the University of Rochester School of Medicine and Dentistry. Later, he joined the faculty of a newly formed medical school, Mercer University School of Medicine (MUSM), in Macon, Georgia, where he is currently a full professor.

James Koropatnick obtained his Ph.D. in genetics at the University of British Columbia (UBC) in 1980 followed by a postdoctoral Alberta Heritage Medical Research Foundation Fellowship at the University of Calgary until 1984. He came to the University of Western Ontario (UWO) and the Lawson Health Research Institute in London, Ontario, Canada in 1985 and is now a Distinguished Oncology Scientist, director of the LRCP Cancer Research Laboratory Program, director of the UWO/Canadian Institutes of Health Research (CIHR) Strategic Training Program in Cancer Research and Technology Transfer, assistant director of the Lawson Health Research Institute, and a full professor in the UWO Department of Oncology with cross-appointments in the UWO Departments of Microbiology and Immunology, Physiology and Pharmacology, and Pathology. He is also the chief scientific officer of Sarissa, Inc. (a biotech discovery company) and sits on the Scientific Advisory Boards of Critical Outcomes Therapeutics, Inc., ID Laboratories, Inc., and MedVax Pharma, Inc.

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1 Cellular Inorganic Chemistry Concepts and Examples

David H. Petering, Rajendra Kothinti, Jeffrey Meeusen, and Ujala Rana

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1.1 INTRODUCTION

Cells and organisms require many different metal ions. As many as 3000 proteins in the human proteome utilize Zn^{2+} for structural or catalytic purposes [1,2]. Hundreds of proteins employ iron and copper [3,4]. When these and other metal ions are unavailable nutritionally or their metabolism is deranged, the consequences can be severe.*

Several nonessential, toxic metals consistently appear in the list of environmental pollutants of most concern for human health [5–7]. Still, some of the best anticancer therapeutic agents are metallodrugs or otherwise interact with metals as part of their mechanism of action [8–11]. Nevertheless, despite the challenges and opportunities, surprisingly few scientists study metal ions in biological systems. Fewer still focus on questions in metallobiology from a chemical perspective, striving to link chemistry with biology. Thus, physiological or pathological studies may conclude that a metal ion or complex *causes* a particular cellular outcome and delineate changes that ensue upon perturbation of the metallic species but never define the actual site where the inorganic chemistry takes place. For example, zinc deficiency *causes* defects in immune response, *causes* apoptosis, and *inhibits* cell proliferation, but the molecular sites that undergo depopulation of Zn^{2+} and start complex cascades of reactions leading to these outcomes are largely unknown [12–14]. Or, Pb^{2+} and CH_3Hg^+ *induce* neurotoxicity that exhibits well-established phenotypes [15,16]. However, relatively little is known about the specific binding sites occupied by these ions and how such interactions initiate and perpetuate toxicity.

This chapter offers an excursion into metallobiochemical research aimed at revealing the importance of the chemical perspective for studying and understanding metallobiological processes. The topics reflect the authors' interests in relation to subjects addressed in this monograph. The discussion begins with a general introduction to inorganic reaction classes. Then several topics are used to illustrate a combined chemical-cellular approach to investigating metallobiological problems related to metal ion metabolism.

1.2 INTRODUCTION TO INORGANIC BIOCHEMISTRY RELATED TO METAL ION TRAFFICKING

Cells present themselves to researchers as remarkably complex, endlessly integrated entities. Until recently, biochemists gained information and understanding about cellular chemistry by studying individual metabolic reactions and cellular structures. As new technologies emerged, scientists began studying collectives such as the genome and the proteome, with the aim of comprehending how cell structures interact and work together to generate the basic living system, the cell.

The "omics" perspective now extends to virtually any grouping of molecules within the metabolome (all of the metabolites in the cell), including the glycosylome, the lipidome, and the *metalome* [1–4]. At first sight, one wonders what rationale might justify grouping diverse metal ions into the *metalome*. In a sentence: All are small, positively charged ions that are *metabolized* by a small set of general inorganic reaction mechanisms.

Metabolism means the collection of reactions that govern the organized cellular uptake, distribution, and efflux of metal ions (M) that link their presence in cells to their localization in specific sites, where they participate in a huge array of structures and reactions. Used in this way, the *metabolism* of metal ions is called *trafficking*. As charged entities, metal ions exist in aqueous

* Abbreviations: CA, carbonic anhydrase C; DEA/NO, diethylamine nonoate; DTNB, 5,5*N*-dithio-bis(2-nitrobenzoate); EGTA, (2,2′-oxypropylene-dinitrilo)tetracetic acid; FRET, fluorescent resonance energy transfer; green fluorescent protein; ICPMS, inductively coupled plasma mass spectrometry; MT, metallothionein; PAGE, polyacrylamide gel electrophoresis; PYR, pyrithione, 2-mercaptopyridine-*N*-oxide; SNAP, *S*-nitrosyl-acetylpenicillamine; TPEN [*N,N,NNNN*-tetrakis(2-pyridylmethyl)-ethylenediamine]; TSQ, *N*-(6-methoxy-8-quinolyl)-*p*-toluensulfonamide.

solution either in aquated form neutralized by an equivalent number of negatively charged ions or as complexes with charged or polar ligand molecules that bind metal ions through electron-rich metal ion binding sites involving N, O, and/or S atoms. Trafficking of biologically essential metal ions from outside the cell to the final sites of functional activity such as metalloproteins consists conceptually of a series of directed reactions that involve metal-ligand species at every step along each pathway.

The grand conceptual problem in metal ion trafficking may be posed as follows:

A substantial number of metal ions or metallic species play key roles in cellular processes. Their properties range from those of alkali metal ions to the left of the periodic table to transition metal ions such as $Fe^{2+,3+}$, Zn^{2+}, and $Cu^{1+,2+}$. In cells, they confront a multitude of metal ion binding ligands, both their natural binding sites and many other potential sites that compete for binding. The latter exist simply because proteins (amine, imidazole, carboxyl, and thiol groups) and nucleic acids (phosphate and base nitrogen and oxygen substituents) are replete with groups that display significant affinity for metal ions. In this heterogeneous environment, how are specific pathways that deliver metal ions from outside the cell to their ultimate binding sites favored?

The entrance into the cell and the pathological activity of toxic or therapeutic metal ions or metal complexes, such as Cd^{2+}, Pb^{2+}, and *cis*-diamminedichloro-Pt(II), must also be based on similar principles of metal ion trafficking, involving intracellular binding sites and the formation of metal-ligand complexes that are not normally part of the cellular milieu.

1.2.1 Metal-Ligand Binding

The generalized trafficking reactions consist of the following processes, in which M and L are metal ion and metal binding ligand, respectively [17]. Each reaction is characterized by an equilibrium (stability) constant (K) and rate constants (k_1, k_{-1}) for the forward and reverse reactions:

$$\mathrm{M} + \mathrm{L} \rightleftharpoons \mathrm{M\text{-}L}, \quad K, k_1, k_{-1}, \tag{1.1a}$$

$$\mathrm{iM} + \Sigma \mathrm{L_i} \rightleftharpoons \Sigma \mathrm{M\text{-}L_i}. \tag{1.1b}$$

Reaction 1.1a describes the association of the metal ion with a ligand. This reaction and its equilibrium constant, K, and rate constants for formation and dissociation, k_1 and k_{-1}, comprise fundamental information about biological M-L complexes that can be used to assess the comparative energetic favorability of binding sites for particular metal ions and the kinetic stability of the product complexes. As one moves from left to right in the periodic table, metal ions progressively prefer to bind to oxygen, then nitrogen, and finally sulfhydryl ligands [18]. The same trend operates on moving down the table within elemental families.

Alkali (Na^+, K^+) and substantial concentrations of alkaline earth (Mg^{2+}, Ca^{2+}) metal ions exist in cells as free metal ions because the equilibrium constants with cellular ligands are relatively small to modest and the rates of formation (k_1) and dissociation (k_{-1}) are rapid [19]. As such, the succession of formation and dissociation reactions, conceived for a variety of ligands (ΣL_i, sum of many intracellular ligands, Reaction 1.1b), constitutes a primary means of distributing M among binding sites ($\Sigma M\text{-}L_i$) according to equilibrium stability. In contrast, for transition metal ions such as $Fe^{2+,3+}$, Zn^{2+}, or $Cu^{1+,2+}$ and toxic, heavy metal ions including Cd^{2+}, Hg^{2+}, or Pb^{2+}, the concentration of free metal ion may be vanishingly small because cells contain many natural metal ion binding sites with large equilibrium constants for M as well as an abundance of lower affinity sites that, nevertheless, represent a very large combined affinity for M [20]. In this situation, it becomes paramount to understand the mechanisms by which native metal ions (metal-ligand complexes) reach specific sites and toxic metal ions either localize selectively or distribute nonspecifically within the cell.

1.2.2 Ligand Substitution

$$\text{M-L} + \text{L} \rightleftharpoons \text{M-L} + \text{L}, \quad K, k_1, k_{-1}, \tag{1.2a}$$

$$\text{M-L} + \Sigma\text{L}_i \rightleftharpoons \text{L} + \Sigma\text{M-L}_i, \quad K, k_1, k_{-1}. \tag{1.2b}$$

Reaction 1.2a represents the most general means by which transition and toxic metal ions move from one site to another. For transition metal ions, M-L complexes that have large equilibrium constants (Reaction 1.1a) may still be kinetically reactive in Reaction 1.2a (large k_1). As such, their Rate of distribution among ligands would not be rate limited by small dissociation rate constants in Reaction 1.2a, implied by the large thermodynamic stability of M-L or the inherent inorganic properties of the metal ion. For example, the documented trafficking of Cu from cell membrane to metalloprotein binding site is characterized by a series of ligand substitution reactions that successively transfer Cu(I) from one thermodynamically stable binding site to another (Figure 1.1) [21]:

$$\text{Cu(I)-L}_1 + \text{L}_2 \rightleftharpoons \text{Cu(I)-L}_2 + \text{L}_1, \tag{1.3}$$

$$\text{Cu(I)-L}_2 + \text{L}_3 \rightleftharpoons \text{Cu(I)-L}_3 + \text{L}_2, \tag{1.4}$$

$$\text{Cu(I)-L}_3 + \text{apo-protein} \rightarrow \text{Cu(I)-protein} + \text{L}_3. \tag{1.5}$$

Each of these reactions must be thermodynamically favorable and kinetically feasible. Moreover, since each Cu-protein terminates a specific pathway of copper trafficking, there would seem to be kinetic barriers to interpathway Cu(I) transfer as in Reaction 1.6:

$$\text{Cu(I)-L}_{1,\text{pathway 1}} + \text{L}_{2,\text{pathway 2}} \rightleftharpoons \text{Cu(I)-L}_{2,\text{pathway 2}} + \text{L}_{1,\text{pathway 1}}. \tag{1.6}$$

A particularly stringent test of the forbidden nature of such reactions occurs when the metal binding protein metallothionein is present in cells as a metal-unsaturated protein (apo-MT) [22]. The very large affinity of apo-MT for Cu(I) suggests that Reaction 1.6 is thermodynamically favorable when $\text{L}_{2,\text{pathway 2}}$ represents apo-MT. Yet, the metal-unsaturated pool of MT contains little, if any, Cu(I). Nor does its presence seem to perturb Cu metabolism.

Ligand substitution reactions also provide a general route by which nonessential metal ions and metal ion complexes, either toxic contaminants or pharmacological agents, gain access to target molecules and, on binding to them, modify their biological activity. On examining the Hg^{2+} and CH_3Hg^+ stability constants with molecules containing N, O, and S ligating groups, each species displays enormous preference for sulfhydryl group-containing ligands [23]. Nevertheless, the

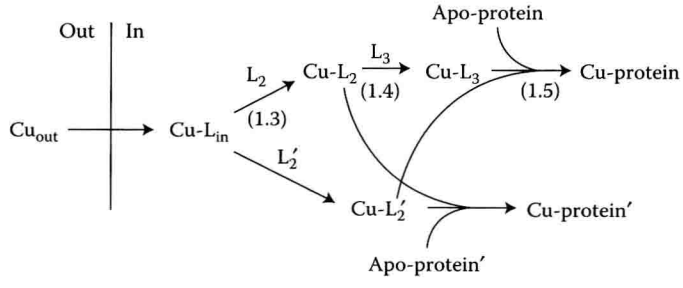

FIGURE 1.1 Generalized copper trafficking by ligand substitution with chaperones (L). Numbers in parentheses refer to reactions in text.

affinities of an array of sulfhydryl ligands for mercury are similar and the ligand substitution rates are rapid [24]. Thus, CH_3Hg^+ readily distributes among competing sulfhydryl-containing sites:

$$CH_3Hg\text{-}SR + R'SH \rightleftharpoons CH_3Hg\text{-}SR' + RSH. \tag{1.7}$$

In this case, mercurial localization must depend on other factors such as the contribution of the methyl group to the equilibrium or kinetic stability of CH_3Hg-SR′ species.

Similarly, the antitumor drug *cis*-dichlorodiammine Pt(II) reacts with DNA guanine bases through ligand substitution reactions, leading to cytotoxic DNA adduct species [25,26]:

$$(NH_3)_2PtCl_2 \rightleftharpoons (NH_3)_2PtCl(H_2O) \rightleftharpoons (NH_3)_2Pt(H_2O)_2, \tag{1.8}$$

$$(NH_3)_2Pt(H_2O)_2 + \text{N-N (adjacent } N^7\text{-nitrogens of guanine)} \rightarrow (NH_3)_2Pt(\text{N-N}). \tag{1.9}$$

In this case, the rate limiting reactions are the dissociation of Cl^- ions, followed by the rapid substitution of guanine nitrogens for bound water molecules. Studies of the reactivity of the platinum drug with alternative ligand binding sites in the cell, for example, demonstrate that *cis*-dichlorodiammine Pt(II) reacts faster with metallothionein than with DNA because the thiolate compound can directly attack the dichloro species [27–29]. Thus, mechanisms of drug resistance may involve sulfhydryl-containing molecules such as metallothionein or glutathione that react with the drug and inactivate it toward further reaction with DNA or other sites [30,31]:

$$(NH_3)_2PtCl_2 + 2RS^- \rightleftharpoons (NH_3)_2Pt(SR)_2 + 2Cl^-. \tag{1.10}$$

1.2.3 Metal Ion Exchange

$$M_1 + M_2\text{-}L_2 \rightleftharpoons M_1\text{-}L_2 + M_2, \quad K, k_1, k_{-1}, \tag{1.11a}$$

$$M_1\text{-}L_1 + M_2\text{-}L_2 \rightleftharpoons M_2\text{-}L_1 + M_1\text{-}L_2. \tag{1.11b}$$

Metal ion exchange Reactions 1.11a and 1.11b represent a class of reactions that essential metal ions must and do avoid during trafficking so that the selective binding of specific metals to particular sites is achieved. But in the face of exposure to toxic metal ions, this type of reaction becomes a primary consideration. Competition between essential and toxic metal ions for physiologically important metal ion binding sites is thought to comprise a major category of reaction leading to cell injury. Thus, acute Pb^{2+} exposure in humans *causes* anemia due to the lack of protoporphyrin IX for heme synthesis and hemoglobin formation [32]. Pb^{2+} or a Pb-ligand complex inhibits δ-amino-levulenic acid dehydratase (ALAD) by displacing active site Zn^{2+} from the enzyme, resulting in a Pb-enzyme that is inactive and unable to participate in porphyrin synthesis [33]:

$$Pb^{2+} + \text{Zn-ALAD} \rightleftharpoons \text{Pb-ALAD} + Zn^{2+}. \tag{1.12}$$

In the case of Cd^{2+}, its metal ion exchange reaction with Zn-metallothionein (Zn-MT) serves as the primary means to protect cells from Cd^{2+} toxicity [34–36]:

$$Cd^{2+} + \text{Zn-MT} \rightleftharpoons \text{Cd-MT} + Zn^{2+}. \tag{1.13}$$

Indeed, in some cases, Cd-substituted Zn-proteins can undergo direct metal ion exchange with Zn-MT, resulting in reactivation of the Cd-impaired protein, as has been seen in the case

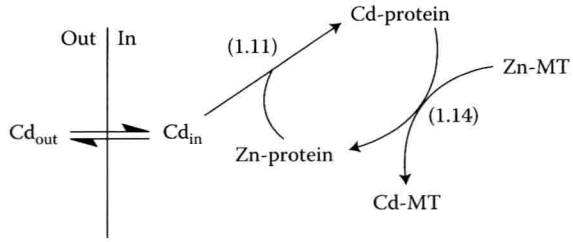

FIGURE 1.2 Metal ion exchange between metalloprotein and metallothionein. Numbers in parentheses refer to reactions in text.

of a Cd-modified Zn-finger protein, tramtrack, and Cd-carbonic anhydrase (CA) (Figure 1.2) [37,38]:

$$\text{Cd-tramtrack} + \text{Zn-MT} \rightleftharpoons \text{Zn-tramtrack} + \text{Cd-MT}. \tag{1.14}$$

1.2.4 Adduct Formation

$$\text{M-L} + \text{L}' \rightleftharpoons \text{L}'\text{-M-L}, \quad K, k_1, k_{-1}. \tag{1.15}$$

Reaction 1.15 symbolizes the association of metal ion binding ligands with metal ion centers of M-L complexes to form ternary complexes. The importance of this type of reaction for cellular chemistry remains to be seen. Nevertheless, the presence of millimolar concentrations of glutathione with its prominent sulfhydryl group begs the question of whether it interacts with metalloprotein metal binding sites that are ligand unsaturated (e.g., Zn-CA).

Considering the reactions of some xenobiotic metal complexes or metal ion binding ligands with cells, the formation of adduct species is an attractive means of bringing these species into association with particular sites and molecules in the cell. For instance, in the reaction of pyridoxalthiosemicarbazonato-Cu(II) (Cu(II)-PTSC) with cells, electron spin resonance (ESR) spectroscopy provides clear evidence that the metal complex initially forms an adduct species and then undergoes redox chemistry that may account for its strong cytotoxic behavior [39]:

$$\text{Cu(II)-PTSC} + \text{L}' \rightarrow \text{L}'\text{-Cu(II)-PTSC}. \tag{1.16}$$

The cellular adduct can be modeled by GS-Cu(II)-PTSC, in which GS is glutathione. Once formed, it may undergo internal oxidation reduction, resulting in the formation of GSSG and Cu(I)-PTSC that reacts with O_2 to initiate the production of reactive oxygen species and regenerate Cu(II)-PTSC for further reaction with the reduction equivalents of the glutathione pool.

1.2.5 Redox Reaction

$$\text{M-L}_{\text{oxidized}} + \text{C}_{\text{reduced}} \rightarrow \text{M-L}_{\text{reduced}} + \text{C}_{\text{oxidized}}, \quad K, k_1, k_{-1}. \tag{1.17}$$

The redox reactions generalized in Reaction 1.17 play key roles in the chemistry of metal ions with multiple, accessible oxidation states such as $Fe^{2+,3+}$ and $Cu^{1+,2+}$ as well as metal complexes that involve redox-active thiolate ligands. In this context, unregulated oxidation–reduction reactions