1
The Position of Metallomics Within Other Omics Fields

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1.1
Introduction

Modern analytical techniques, which were developed during the past decades, allowed a remarkable progress of knowledge in biology, biochemistry, and life sciences. The aim of these new technologies is to acquire – ideally – complete sets of molecular data of the genome, proteome, transcriptome, and metabolome. The suffix *-ome* refers to the entirety of, for example, genes, proteins, or metabolites in a regarded system while *-omics* corresponds to the related scientific investigations for acquiring global qualitative and quantitative information on the respective objects. Thus, terms such as *genomics*, *transcriptomics*, *proteomics*, and *metabolomics* were coined. These "-omics" fields have the ambitious aim to integrate genome, transcriptome, proteome, and metabolome data in order to expand our knowledge on organisms or ecological systems. Such integration and interpretation of large datasets improve the understanding of pathway functions and regulatory networks. This includes large-scale, high-throughput experiments and computational and theoretical approaches in order to advance the frontier of knowledge on biological systems.

Metals play an important role in many life processes; on the one hand, they can be essential, on the other hand, metals can be toxic. In analogy to the previously mentioned fields, the term *metallome* was coined to describe the entirety of metal and metalloid compounds in an organism or its parts (cells, body fluids, or tissues). The study of the metallome and thus the related scientific investigations necessary to acquire and integrate metallome data were denominated by the term *metallomics*.

1.2
Metallome and Metallomics in Relation to Other "-Ome" and "-Omics" Fields

Metallomics is not an isolated research field, it has to be regarded rather in relation to other "-ome" and "-omics" fields in order to explain the specificity of

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metallomics. The following subsections give a very brief overview on genomics, transcriptomics, proteomics, and metabolomics and their specific scientific aims [1] in order to position the role of metallomics within these research fields.

1.2.1 Genomics

The genome is the entirety of genetic information of an organism necessary for its development and functioning. This information is encoded in the deoxyribonucleic acid (DNA). The genome is usually regarded being static.

Genomics aims to answer the following question: *What is the nucleobase sequence of the DNA?*

The analytical tools for genomics are developed to determine the whole DNA sequence of an organism and to enable genome mapping on a fine scale by assigning DNA fragments to chromosomes. These tools (e.g., chain termination, gel electrophoresis, next-generation sequencing platforms, polymerase chain reaction) are analytical key techniques in molecular biology.

1.2.2 Transcriptomics

For the development and functioning of an organism, the genetic information has to be expressed. The first step of the expression of genes is their transcription to ribonucleic acid (RNA) molecules. More precisely, the genetic information from DNA is transcribed to messenger RNA (mRNA). This is followed by the second step, the synthesis of proteins encoded in RNA. Most RNA molecules are single-stranded and much shorter than double-stranded DNA. During transcription, the produced RNA molecule represents a copy of the DNA molecule in the expressed gene and carries the genetic information for the synthesis of proteins.

The transcriptome represents the entirety of all RNA molecules in a biological system (e.g., a cell) at a particular time. Therefore, in contrast to the genome, the transcriptome is highly dynamic because genes that are actively expressed can vary with environmental conditions. In addition to the mRNA encoding for proteins, the transcriptome includes ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), and other noncoding RNA.

Transcriptomics studies global gene expression at the RNA level and thus aims to answer the following question: *Which genes are actively expressed?*

1.2.3 Proteomics

The proteome is the entirety of proteins in an organism, a tissue, a cell, or a body fluid. Proteins are biopolymers that are essential parts of organism and participate in almost every biological process within cells. They consist of a sequence of amino acids, which are linked by peptide bonds determining the

three-dimensional structure and the functions of a protein. The amino acid sequence is encoded in the genome and translated from the nucleobase sequence in the mRNA where a sequence of three bases is encoding one amino acid. However, the same DNA sequence in the genome can result in hundreds of different proteins and their modifications. During transcription from the DNA, alternative splicing leads to several mRNAs. Furthermore, after translation from mRNA to proteins, enzymatic reactions can result in posttranslational modifications such as phosphorylation, glycosylation, and acetylation. Moreover, several single proteins can form protein–protein complexes with new functions. All this shows that the proteome is more complex than the genome and the transcriptome. It is highly dynamic, depends on different factors such as the physiological state of a cell and its environmental conditions, and varies with time.

Proteomics aims to answer the following question: *Which proteins are synthesized?*

Answering this question includes the global study of identity, structure, quantity, and function of proteins while respecting the highly dynamic character of the proteome. Moreover, proteomics is not limited to deciphering amino acid sequences of proteins, thus their primary structures. Rather it also involves the study of tridimensional protein structures, which determine the protein function and which can change in time.

1.2.4 Metabolomics

The metabolome denotes the entirety of all metabolites in an organism. Metabolites are intermediates or end products of physiological processes, usually small molecules with molecular weights below 1 kDa. Similarly to the transcriptome and proteome, the metabolome is highly dynamic and changes its composition each moment, containing a plenty of different metabolites. For example, in plant biology, plants produce a huge number of metabolites, more than most other organisms. The structural diversity of metabolites in the plant kingdom is enormous; the total number of structures is estimated to be up to 5 000 000.

The analytic of metabolites has a long history, starting in medicine in order to get information on diseases of patients. For example, ancient Chinese doctors used ants for the detection of the metabolite glucose in the body fluid urine for the diagnosis of diabetes. In the Middle Ages, urine was investigated for color, taste, and smell, which change with the containing metabolites. Nowadays, metabolomics is one of the key technologies in life sciences and biology.

The main objective of metabolomics is a global identification and quantification of all metabolites in a biological system (cell, fluid, tissue) including their time- and space-resolved distribution. This is analytically the most challenging approach, which is distinguished from other strategies in metabolome research. Metabolite target analysis aims to quantify only one or few target metabolites. Metabolomic profiling is intended to describe qualitatively and quantitatively metabolic pattern for a group of related metabolites. In contrast, metabolic

fingerprinting performs high-throughput sample screening and allows thus their rapid classification without identification and quantification of each individual metabolite.

Thus, metabolomics aims to answer the following question: *Which metabolites are produced in cellular processes?* This includes their structural identification and their quantification as well as the question how the metabolome is linked to the genome, transcriptome, and proteome, for example, the study of genotype–phenotype relations.

1.2.5 Metallomics

The metallome represents the entirety of metal and metalloid compounds in an organism or its parts (cells, body fluids, or tissues) [2, 3]. In addition to the other "-ome" fields, investigation of the metallome highlights the importance of essential and toxic metals. The regarded metal and metalloid compounds are formed during (or involved in) physiological processes, which include DNA, RNA, proteins, and metabolites. Therefore, metallomics is interrelated to the other "-omics" fields and cannot be regarded without them.

Metallomics aims to answer the following question: *What are the interactions and functional connections between metal ions and DNA, RNA, proteins, and metabolites?* This includes a global structural identification and quantification of metallobiomolecules as well as their link to the genome, transcriptome, proteome, and metabolome.

Interactions of metals with DNA and RNA play important roles for basic structural and kinetic aspects but also for medical applications. An important example for the latter point are anticancer platinum drugs; for example, cisplatin forms monoadducts with DNA causing DNA cross-linking, which inhibits DNA repair or DNA synthesis in cancer cells [4, 5].

Most interactions occur between metals and proteins or metabolites. It is estimated that more than 30% of all proteins contain metals [6]. Many proteins need metals as cofactor to be functional. Examples are Fe in hemoglobin as well as Cu and Zn in superoxide dismutase. Metallothioneins, a family of small, cysteine-rich proteins, can bind essential as well as toxic metals such as copper, zinc, cadmium, mercury, arsenic, and silver. All these metalloproteins are part of the proteome but can also be regarded as part of the metallome. Also, metabolites can contain metals or interact with metals forming new metal compounds. For example, in plants, an important metabolite is glutathione having a thiol group, which can bind to metals. Furthermore, phytochelatins, derivatives of glutathione with several thiol groups, are synthesized in plants upon toxic metal stress and can form complexes with these metals [7]. These compounds can be regarded as part of the metabolome as well as part of the metallome.

The boundary between the metallome and the other "-ome" fields seems to be blurred. In order to systematize this situation, the metallome can be regarded

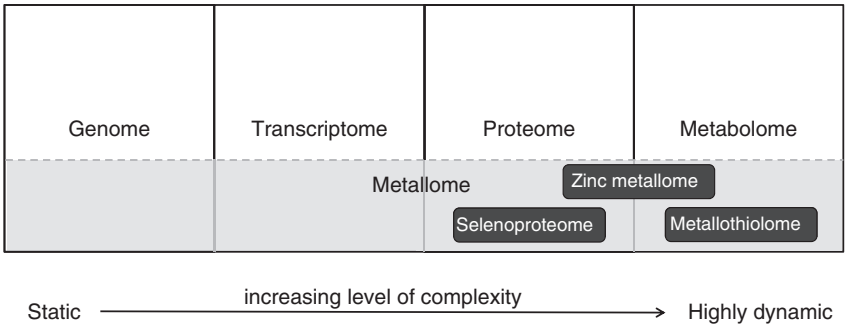

Figure 1.1 The metallome as subcategory of the genome, transcriptome, proteome, and metabolome. Examples for subgroups of the metallome.

as subcategory of the genome, transcriptome, proteome, and the metabolome (Figure 1.1) with the specificity that metals interactions with DNA, RNA, proteins, and metabolites are included. At this point, the following question arises: Can metallomics be a separate research field or should it be integrated into the other "-omics" fields? Metallomics has its own specificity by focusing on metal compounds within organisms. The study of the metallome requires specific analytical strategies and therefore metallomics can be considered as own scientific discipline, which is, however, strongly interrelated to genomics, transcriptomics, proteomics, and metabolomics.

1.3
Is Metallomics Feasible as a Global Study of the Metallome

The complexity of the metallome increases from metal–DNA to metal–metabolite interactions. In consequence, similarly to the proteome and metabolome, the entire metallome is also highly dynamic, changing each instant. When regarding the already enormous number of proteins and metabolites in an organism and then adding possible metal interactions with these molecules, the metallome comprises an immense amount of diverse structures in permanent transformation. This includes not only thermodynamically stable metallobiomolecules but also labile complexes and intermediates. All this demonstrates that the analytical challenges are enormous and that the available analytical techniques today can only approach, but not fully accomplish, a comprehensive metallome analysis. However, with respect to the other fields, the suffix *-omics* always implies a *global* investigation of the regarded objects, that is, of genes, proteins, or metabolites. As a consequence, it can be stated that so far metallomics in its primary meaning as a global study of the entire metallome, that is, the entirety of metal and metalloid compounds, is still rather a vision than a realizable concept.

1.4 Approaching the Metallome: Study of Metallome Subgroups

The previous section described the dilemma between the claim of metallomics as the global study of the metallome and the reality given by the limitations of available analytical techniques in view of the complexity of the samples. How to approach the metallome in this situation? As a consequence of the analytical limitations, metallomics research is focused to date on subgroups of the metallome (Figure 1.1), which is more feasible than a global metallome study. Subgroups of the metallome can be metallobiomolecules of a specific element, for example, the zinc or the iron metallome. Subgroups can also be groups of selected metalloproteins or metallometabolites. Example is the investigation of selenoproteins or arsenolipids. Another possibility is the study of specific group of ligands and their metal complexes, for example, metal complexes with thiol peptides. This subgroup of the metallome has been denoted as metallothiolome and its investigation as metallothiolomics [7].

1.5 Analytical Strategies in Metallomics

The following subsections give an overview of analytical strategies for metallomics. Many of them are described in detail in the subsequent chapters of this book.

1.5.1 Element Mass Spectrometry (ICP-MS)

As in metabolomics, the analytical goal in metallomics is the identification, quantification, and localization of compounds. A first approach in metallomics studies is the detection of all metals and metalloids present in the sample and the determination of their total concentration. The method of choice is inductively coupled plasma–mass spectrometry (ICP-MS), a technique for ultrasensitive detection and quantification of chemical elements [8, 9]. For global trace element analysis including biologically significant nonmetals (e.g., chlorine, bromine, iodine), the term *ionomics* is applied [10].

1.5.2 Coupling Techniques

Techniques such as liquid chromatography (LC) and capillary electrophoresis (CE) serve for the separation of metallobiomolecules. For their detection and identification, LC and CE are coupled to mass spectrometry (MS) similarly in proteomics and metabolomics [9, 11]. Special attention has to be paid to the stationary phase of the LC, which should not affect the metal–biomolecule complex.

Size-exclusion chromatography is often used as soft separation technique, but its chromatographic resolution is low allowing rather a fractionation of the sample than a separation of the compounds. A specificity in metallomics is that LC (and CE) is coupled additionally to ICP-MS. This allows a specific, highly sensitive detection of metallobiomolecules in a sample via the metal signal in ICP-MS. Liquid chromatography–inductively coupled plasma–mass spectrometry (LC-ICP-MS) is highly selective for metal compounds while other biomolecules are not detected. Furthermore, LC-ICP-MS enables quantification of metallobiomolecules by their metal signal, provided that these compounds have been structurally characterized. However, structural identification and characterization are only possible by molecular MS. Thus, the complementary application of LC-ICP-MS and LC-MS is the workhorse in metallomics [9]. Furthermore, one important limitation is that only those metallobiomolecules can be analyzed, which are stable during the analytical procedure including sample preparation and separation. This, however, excludes a certain number of kinetically and thermodynamically labile metal–biomolecule complexes of the metallome from being analyzed by these methods. In certain cases, CE is applied as separation technique in order to cope with more labile complexes. Another problem of labile compounds is the potential formation of artifacts, which are detected instead of the metallobiomolecule originally present in the sample.

A specific coupling is the coupling of laser ablation with inductively coupled plasma–mass spectrometry (LA-ICP-MS) [12]. Besides element imaging (see next paragraph), this technique can be applied to gel electrophoresis for the detection of metals in protein spots. However, if metalloproteins are targeted to be analyzed by this method, nondenaturating gel electrophoresis, instead of SDS-PAGE, has to be used, because protein denaturation would destroy the 3D protein structure and thus releases the metal cofactor.

1.5.3
Elemental Imaging Techniques

Techniques for imaging of metals and other concomitant elements (e.g., S and P) approach the local distribution of the metallome in a cell or tissue.

Laser-ablation ICP-MS allows imaging of metals in biological samples with a resolution down to 4–20 μm, which is suited to localize metals in tissues [12–14]. However, the resolution is not sufficient to investigate metal distribution at cellular or even subcellular level.

Third-generation synchrotron facilities are providing beamlines with high flux X-ray beams focused down to a size of about 50 nm [15]. This allows micro X-ray fluorescence (μXRF) and X-ray absorption spectrometry (XAS) at single-cell level including micro extended X-ray absorption fine structure (μEXAFS) and micro X-ray absorption near-edge structure (μXANES). While XRF enables elemental imaging, EXAFS and XANES provide additional information about the chemical environment of metals and thus their coordination by ligands (e.g., thiol groups). While XANES allows identification of the neighboring atoms, EXAFS provides

even information about the next but one atom. Without complex sample preparation, metals can be investigated directly in their natural environment. Therefore, biological materials, for example, cells, are frozen in liquid nitrogen (−196 °C), fixed on a sample support, and analyzed as frozen hydrated samples.

Nano secondary ion mass spectrometry (NanoSIMS) is another element imaging technique. This technique is also suitable for localization of metals at subcellular level with resolution down to 50 nm [16]. Unlike in X-ray techniques, the sample cannot be analyzed in frozen state but has to be dehydrated and embedded in a resin as for electron microscopy. Other techniques for element-specific imaging include energy-dispersive X-ray spectrometry (EDX) coupled to electron microscopy [17].

Although these imaging techniques provide primarily information about metal distribution with high spatial resolution and additional information of their chemical environment when using EXAFS and XANES; however, structural characterization of metal compounds and thus a global metallomics study are not possible. For detailed information regarding element imaging techniques applied in metallomics, see the chapter on bioimaging (chapter 4).

1.5.4 Bioinformatic Approaches

Experimental data on the metallome acquired with the techniques described earlier can be supported and complemented by bioinformatic approaches. This is mainly applied for the study of metalloproteins for the search of metal-binding sites [18]. Genome and proteome databases can be searched for specific amino acid sequences in proteins, which are prone to bind metals. For example, zinc-binding patterns can be predicted when, for example, Cys_2His_2 (classical zinc finger) or Cys_4 (zinc bundle) sections are found in protein sequences [19]. These sequences are known in Zn-binding zinc-finger proteins. Moreover, bioinformatic calculations can predict metal-binding sites from 3D protein structures [20].

1.6 Functional Connections Between DNA, Proteins, Metabolites, and Metals

As in metabolomics, also in metallomics, the genotype–phenotype relation is an important issue of investigation. The objective is to study which genes are responsible for the presence or absence of particular metals in organisms. These studies are mainly carried out for plants. Therefore, plant wild-type samples are compared for their metal content with mutants where specific genes are knocked out. Thousands of plant samples are analyzed for their trace element content and the metal concentrations are statistically compared. Metals are either globally analyzed in bulk samples by ICP-MS or spatially resolved by imaging techniques where the latter method provides more information than a simple bulk analysis. In this way, empirical connections between genes and metal concentrations/distributions can

be established [10]. This approach, also denoted as ionomics, does not regard the proteins and metabolites involved, which are, however, the link between genes and metals represented by metalloproteins and metallometabolites.

Other approaches try to link the genome to the metalloproteome or the metallometabolome. These methods are similar to those described for gene, protein, and metabolite functional analysis and their data integration [1] extended by metal analysis. For example, the metal hyperaccumulating plant *Noccaea caerulescens* (former name *Thlaspi caerulescens*) was investigated by complementary genome and metabolite analysis (Figure 1.2) [21]. Therefore, a DNA library of the plant was constructed in yeast, that is, a population of yeast cells where each cell is carrying different DNA fragments of the plant. Then, a culture of about 400 000 yeast transformants was submitted to a nickel solution with a Ni^+ concentration, which is lethal for yeast. A few transformants survived the metal toxicity test suggesting that they contain a nickel-resisting gene from the plant *N. caerulescens.* These cells were submitted to DNA sequencing identifying a gene coding of the enzyme nicotianamine synthase. Metal-specific LC-ICP-MS demonstrated the presence of a nickel complex in the surviving yeast clones but also in the original plant. Metabolite analysis by MS identified nicotianamine as ligand suggesting the presence of Ni–nicotianamine in yeast and plant. The analysis of a synthetic Ni–nicotianamine complex confirmed the results. This one of the few examples that shows that integration of genomics and metabolomics with metal analysis leads to the specific identification of metal metabolites.

1.7 Metallothiolomics as Example for Metallomics Studies of a Metallome Subgroup

Specific metallometabolites of particular importance in plants are thiol peptides and their metal complexes involved in the thiol-peptide-regulated metal homeostasis. Intracellular thiol peptides are highly important for biological processes, containing the major active form of sulfur (thiol or sulfhydryl group: –SH) and can serve as ligands for metal binding. Most important are glutathione (GSH) and phytochelatins (PCs). The latter are synthesized in plants under metal stress. Thiol peptides play the most relevant role in the plant and fungi metal homeostasis. Therefore, the entirety of thiol peptides and their metal complexes is referred to as *metallothiolome* and the concept of metallothiolomics can be regarded as a metallomics study of a metallome subgroup. Metallothiolomics summarizes all analytical approaches for the investigation of the thiol-peptide-regulated metal homeostasis [7].

The approaches for the characterization of the metallothiolome are basing primarily on LC-MS coupling systems (Figure 1.3). In most studies, thiol peptide complexes with Cd(II), Pb(II), As(III)/As(V), and Hg(II) are investigated. The fundamental difference is that As– and Hg–thiol complexes are thermodynamically more stable due to the covalent character of As–S and Hg–S bonds. Therefore, different chromatographic conditions are applied for Cd and Pb complexes in

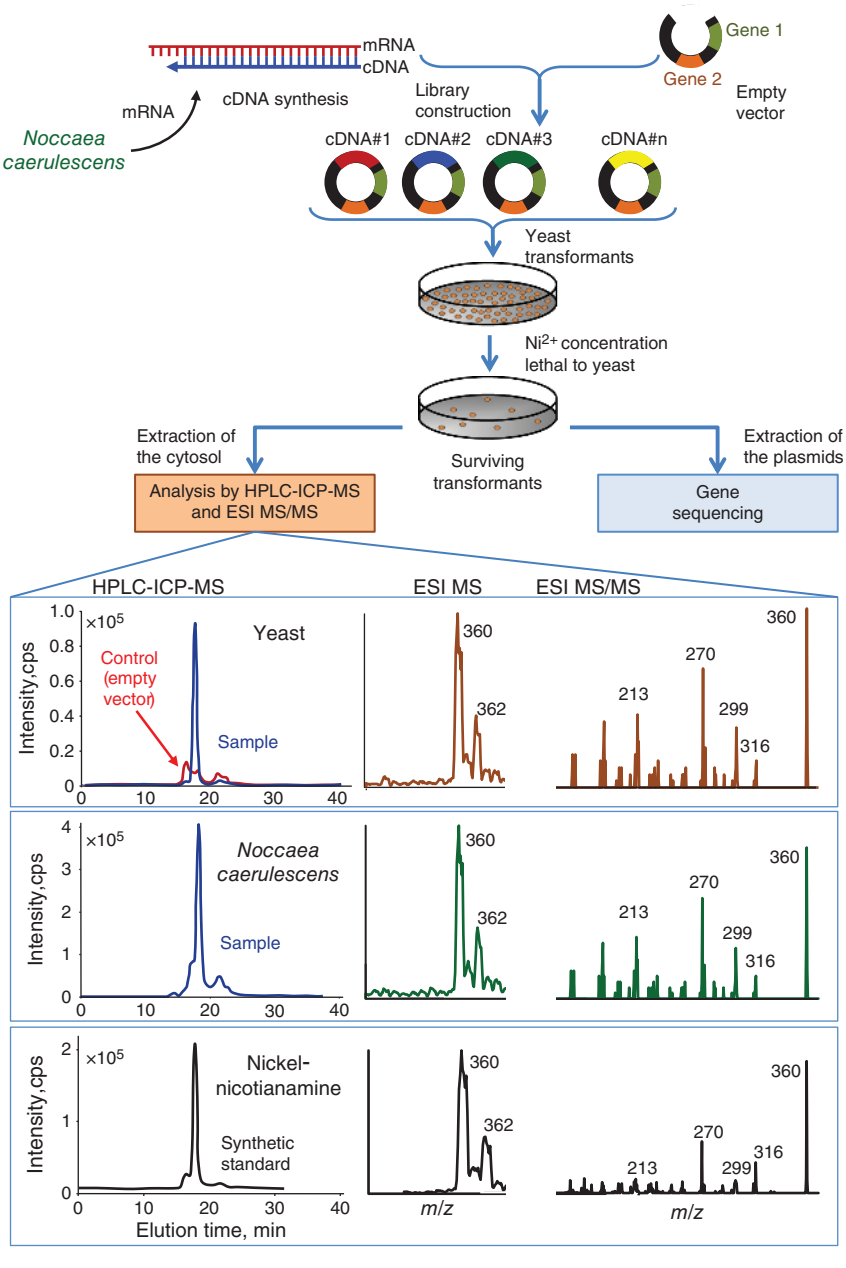

Figure 1.2 Investigation of metal resistance in the metal hyperaccumulating plant *N. caerulescens* by complementary genome and metabolite analysis. (Adapted with permission from [21]. Copyright (2003) American Chemical Society and adapted from [3] with permission of The Royal Society of Chemistry.)

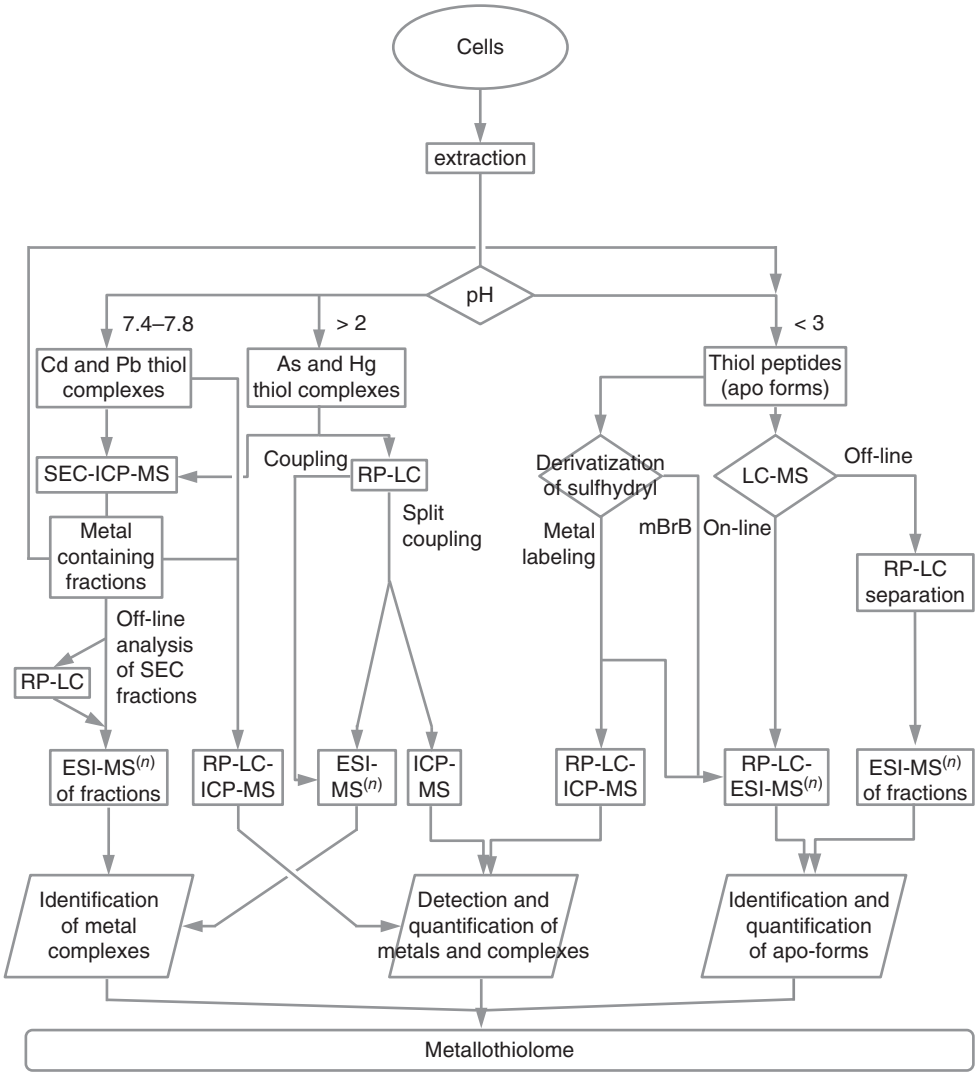

Figure 1.3 Workflow and analytical techniques in metallothiolomics. The metallothiolome is a subgroup of the metallome. (Reprinted from [7], Copyright (2011), with permission from Elsevier.)

contrast to As and Hg complexes. LC-ESI-MS is employed for identification of metal–thiol peptide complexes as well as for identification, structural characterization, and quantification of the metal-free apo-forms. In addition, LC-ICP-MS is used for detection and quantification of metal–thiol complexes.

The example of metallothiolomics demonstrates how progress in analytical developments contributes to revise biochemical models. Classic techniques

for thiol peptide analysis use chromatography with spectrophotometric or fluorescence detection, which can lead to misleading signals. Based on data from these methods, phytochelatins were formerly described with a maximum number of 11 γ-Glu-Cys units. However, only MS can unambiguously identify and accurately quantify thiol peptides eluting from a chromatography column. Therefore, with the new metallothiolomics concept basing on LC-MS methods, the phytochelatin structure was revised having a maximum number of six γ-Glu-Cys units [22].

The study of the metallothiolome demands not only the characterization of the thiol peptide apo-forms but also their native metal complexes. The latter point is still a challenge for analytical chemistry. Therefore, the description of biochemical mechanisms of metal homeostasis including native metal–thiol peptide complexes is still an unsolved issue requiring novel analytical approaches for metallothiolomics and thus in metallomics.

1.8 Concluding Remarks

The metallome as the entirety of metal and metalloid compounds in an organism or its parts (cells, body fluids, or tissues) results from the interactions and functional connections between metal ions and DNA, RNA, proteins, and metabolites. Therefore, the metallome has to be regarded as subcategory of the genome, transcriptome, proteome, and metabolome. Metallomics as investigation of the metallome highlights the importance of essential and toxic metals and metalloids and can therefore be regarded as own scientific discipline, which is, however, strongly interrelated to the other "-omics" fields. Thus, metallomics studies require not only the identification and quantification of metal compounds but also the investigation of their functional connections between DNA, proteins, and metabolites. The metallome is highly dynamic and comprises an immense amount of diverse structures in permanent transformation. Therefore, metallomics in its primary meaning as a global study of the entire metallome is so far not feasible due to the limitations of the available analytical techniques. To date, metallomics focuses on the study of subgroups of the metallome such as selenoproteins and selenometabolites, arsenic, mercury, manganese, and iron species as presented in the different chapters of this book. However, we have to bear in mind that this strategy allows only an approximation to the metallome. With respect to other -omics fields, the term *metallomics* should also imply a global investigation, in this case, of all metal(loid) compounds. The progress in metallomics toward this aim will strongly depend on the development of analytical techniques and the integration of data by bioinformatic approaches.

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