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REVIEW

Transition Metal Speciation in the Cell: Insights from the Chemistry of Metal Ion Receptors

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The essential transition metal ions are avidly accumulated by cells, yet they have two faces: They are put to use as required cofactors, but they also can catalyze cytotoxic reactions. Several families of proteins are emerging that control the activity of intracellular metal ions and help confine them to vital roles. These include integral transmembrane transporters, metalloregulatory sensors, and diffusible cytoplasmic metallochaperone proteins that protect and guide metal ions to targets. It is becoming clear that many of these proteins use atypical coordination chemistry to accomplish their unique goals. The different coordination numbers, types of coordinating residues, and solvent accessibilities of these sites are providing insight into the inorganic chemistry of the cytoplasm.

Ten years ago, several advances in the field of human genetics, namely, the discovery of the genetic basis of Menkes, Wilson, and Lou Gehrig's diseases, began to focus a new spotlight on intracellular metal ion metabolism (*1*–*4*). Before 1993, many metal resistance, uptake, and metalloregulatory systems were known to protect bacteria against transition metal stresses (*5*); however, few metal-binding proteins were known to be directly involved in intracellular trafficking of these essential cofactors. Although extracellular proteins such as transferrin conduct Fe into the cell, the rare examples of intracellular metal-handling proteins were best construed as "metal sponges" that protect the cell against potentially toxic metal-based reactions. Notable examples include ferritin, which is expressed under conditions of excess Fe, and metallothionien, which is induced by excess Zn and Cu.

The discoveries of the Menkes and Wilson disease genes and the related yeast proteins (*6*) provided immediate clues to a different physiological function: These genes encoded integral membrane proteins, working in specialized subcellular compartments within the cell, that were postulated to pump Cu(II) ions across lipid bilayers. Since then, investigations of these and related proteins in bacteria, fungi, and mammals have not only extended this model [for instance, showing that these proteins actually traffic Cu(I)], but have also provided the basis for the discovery of diffusible cytosolic partners for the membrane transporters. These are known as metallochaperones; they protect and guide the metal ions through the cytoplasm, ultimately transferring the ions to specific partner proteins. It is becoming clear that both the transporters and the metallochaperone proteins employ atypical coordination chemistry relative to the enzymes that ultimately incorporate the metal as a cofactor. In this overview, we focus on how the unusual Cu and Zn chemistry of several of these new proteins is changing our view of the inorganic physiology of the cytoplasm.

Inorganic Physiology: Boundary Conditions

Cells avidly acquire a variety of transition elements and ultimately employ them in structurally constrained binding sites, where they can carry out regulatory or catalytic roles. These metals are sometimes referred to as trace elements, but from the point of view of the cell, this is a misnomer. Studies of transition metal quotas of *Escherichia coli*, for instance, reveal that individual bacteria concentrate Zn and Fe by several orders of magnitude relative to the concentration in a typical growth medium until they achieve a quota of about 2×10^5 atoms per cell, which is equivalent to a total concentration of about 0.1 mM (*7*). Metals such as Cu and Mn are maintained in the 10 to 100 μM range. Other metals are also concentrated by the *E. coli* cell to a narrow, fixed, total concentration as follows: K and Mg, 10^8 atoms per cell, >10 mM; Ca, Zn, and Fe, 10^5 atoms per cell, ~0.1 mM; Cu, Mn, Mo, and Se, 10^4 atoms per cell, ~10 μM; V, Co, and Ni, low abundance.

Clearly, many transition metals are abundant in the cell, but so are metalloproteins, which correspond to about a third of all structurally characterized proteins. This raises the question of how the cell allocates the correct metals to specific protein sites while avoiding toxic side reactions at such high total metal ion concentrations. It has long been known that many metalloproteins in the test tube are highly specific chelators that acquire the appropriate metal ions via diffusion, collision, and substitution reactions. But do proteins obtain metal cofactors in the cytoplasm through such collisional processes with small-molecule or metal-protein complexes? Or are there "metal ion pools" in which the "free" form of the metal ion is available for incorporation into newly synthesized proteins? These questions raise the issue of chemical speciation: the breakdown of the total metal complement of the cell into various complexes with proteins, nucleic acids, small molecules, free ions, etc. For instance, when considering the copy number of metalloproteins with high metal ion affinities (*8*) and the abundance of small molecules of moderate metal ion affinity, what percent of the total metal quota of the cell might remain free for newly synthesized proteins? As elaborated below, the free metal pool model is not standing the test, at least in the cases of Zn and Cu: Concentrations of the free forms of these ions within the cell are proposed to be too low to allow an apoprotein to acquire these cofactors without accessory factors.

A common language for inorganic chemistry in the cytoplasm. But what exactly does "free metal ion" mean? Many contextual definitions appear in the literature, but perhaps the most useful is one that allows a connection of our understanding of the cytoplasm with our understanding of the energetics of protein-metal interactions. The definition used in chemical kinetics and thermodynam-

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ics is well suited to this task. Consider the case of Zn and EDTA below, where $[Zn]^{2+}_{(aq)}$ represents the free zinc in solution at equilibrium.

$$[Zn(EDTA)]^{2-}_{(aq)} \rightleftharpoons [Zn]^{2+}_{(aq)} + [EDTA]^{4-}_{(aq)} \qquad K_d'$$

$$[ML]_{(aq)} \rightleftharpoons [M]_{(aq)} + [L]_{(aq)} \qquad K_d''$$

By "free" we mean that for the reaction of a metal ion, M, with a protein or a small-molecule ligand, L, the value $[M_{(aq)}]$ represents the concentration of the free hydrated metal ion. The equilibrium of the metal with each ligand is governed by a pH-dependent equilibrium constant, K_d'. Depending on the number of proteins and other cellular components that bind a given metal in the cytosol, and their individual metal-binding equilibrium constants, a thermodynamic postulate is that the cytosol may be kept at a particular concentration of free metal ions—or "buffered"—by a wide variety of biopolymers and small molecules. In comparison to a pH buffering system, this implies that the cell buffers metal ions in such a way that the free metal concentration will remain relatively constant as total metal concentration changes. A variation on this theme uses the term biologically available metal pool instead of free metal pool, which implies that metals complex with abundant small molecules in the cytoplasm. Neither framework, however, provides a mechanism for facile movement of the metal ions out of adventitious sites and into specific ones.

We still do not know enough about how the bulk of metal ions are conducted within the cytoplasm to resolve these issues, but several studies suggest that thermodynamic considerations are only one side of the coin. A key distinction between metalloproteins of various functions may be the metal-protein off rate. This would be a function of the accessibility of the metal-binding site to attack by incoming ligands and of the ability of a protein to accommodate conformational changes and other features that control the rate of metal binding or removal. Clearly, kinetic as well as thermodynamic studies of metal trafficking will be required to address these issues. As we will see below, the coordination chemistry of a few Cu-specific chaperone proteins appears to be poised to alter the rate of metal transfer to or from a potential partner, depending on the nature of the protein-protein docking interactions, thus allowing a kinetic sidestep of the thermodynamic barriers (*9*).

Free metal ions in the cytoplasm? Copper is one of the more toxic of the essential metals, so the proposal that the cellular Cu budget (in *Saccharomyces cerevisiae*, corresponding to 5.2×10^5 atoms per cell) operates on a "no free metal" principle (*10*), employing metal ion chaperone proteins to allocate Cu to some targets, appeals to common sense. But will cells maintain pools of free metal ions for other more abundant and less toxic elements, such as Zn and Fe? One way to investigate this is by interrogating the intracellular monitors that sense the status of the cellular quota and then adjusting the expression of uptake or efflux genes.

In *E. coli*, calibration of two Zn-sensing metalloregulatory proteins, Zur and ZntR, has shown that these proteins switch off expression of Zn uptake machinery or switch on production of Zn efflux pumps when $[Zn(II)]_{free}$ exceeds an extraordinarily low threshold of 0.5 fM (*7*, *11*). Considering the small volume of a cell, this is six orders of magnitude less than one free cytosolic Zn per cell and is inconsistent with the presence of any pool of free Zn in the absence of stress. Taken together, this suggests that the intracellular milieu has an extraordinary chelation capacity for Zn(II). This perspective is corroborated by studies of other Zn metalloregulatory proteins such as ZiaA and SmtB that also exhibit extraordinary Zn affinities (*12*, *13*). Although the full accounting of all Zn species in the cell is not available, we estimate that between 12 and 50% of all the Zn in the cell can be accounted for in the transcription and translation machinery alone. As copy numbers of the 60-plus additional genes that encode known Zn proteins or their homologs are determined, it is possible that there will be an excess of high-affinity Zn-binding sites relative to the number of Zn atoms in the cell. Finally, there are a number of abundant nucleic acids and small-molecule chelators in the cytosol that bind Zn^{2+} and other metals with moderate affinity. Notable examples include the millimolar levels of nucleotides, free amino acids, and glutathione, which substantially extend the chelation capacity of the cell.

If one accepts that the pool of free Cu^+ in the cytosol of yeast and the pool of free Zn^{2+} in the cytosol of bacteria do not exist, then how do the appropriate metal ions and proteins get together? How are these metal-trafficking proteins able to deliver their metals selectively while operating in such a metal-ion-vacuum? In putting these questions to the test, we consider some inorganic chemistry of metal homeostasis machinery in the cell.

Atypical Coordination Chemistry of Metal-Trafficking Proteins

Metal-trafficking proteins must bind their cargo ions tightly enough to prevent adventitious reactions or release of the ions, but this coordination environment must also allow for easy transfer of the metal to the target. In this light, it is not surprising that the trafficking proteins often make use of unusual coordination chemistry relative to many of the downstream target proteins. Although the inorganic chemistry of only a few systems has been characterized to date, each exhibits biologically novel coordination sites and mechanisms.

Cu(I) receptors in the cytoplasm of yeast. Among the best understood intracellular metal-trafficking systems is that of Cu(I) in yeast (*14*). The atypical coordination environment observed here for Cu provides a mechanistic basis for the dual requirements of tight binding and easy transfer of the Cu to the target protein (*15*). The first Cu chaperone, Atx1, was identified by Culotta and co-workers in elegant genetic screens for new oxidative stress genes in *S. cerevisiae* (*16*). With the discoveries of the Atx1's Cu(I) chemistry and its interaction with a homologous domain of a partner protein, a copper chaperone function was established (*18*); a specific antioxidant activity was ruled out (*19*).

Separately, another yeast protein, the Cu chaperone for superoxide dismutase (CCS), was shown to be required for superoxide dismutase activity (*17*). This metallochaperone directly inserts the Cu into the target (*10*); however, the coordination chemistry in this case is not yet clear. Through these studies, in parallel with structural studies of other homologous proteins, the -GMXCXXC- motif (*20*) has emerged as a key element in a highly conserved metal-handling domain (*21*–*26*). Many proteins containing this motif are now known to bind Cu(I) with two cysteines in a low-coordination-number environment that allows for very tight binding but that can nonetheless allow the entrance of a third binding residue or ligand from outside the protein, or domain, itself. Neither environment is like those seen in any other structurally characterized mononuclear Cu(I) protein, which typically exhibit a coordination number of four. In Atx1, an adjacent lysine sterically hinders the approach of a third ligand, but conformational changes, which may be associated with protein docking, could then allow the partner protein, a domain of Ccc2, to access the anionic $Cu(I)(S\text{-}Cys)_2$ site (*21*, *24*, *27*). Ultimately, this domain transfers the Cu into a trans-Golgi compartment where it can be incorporated into multi-Cu oxidases such as ceruloplasm and Fet3, as well as other Cu enzymes such as peptidylglycine-amidating monooxygenase (*28*) (Fig. 1).

Atx1 can obtain its Cu cargo in vitro from the cytoplasmic domain of the high-affinity Cu uptake protein Ctr1 (*29*); however, Ctr1-independent pathways must be involved in vivo (*18*). Recent reviews of these systems reveal further details of Cu trafficking pathways in yeast, pathogenic bacteria, and photosynthetic bacteria, where an unusual thylakoid Atx1 plays a key role in trafficking between compartments (*15*, *30*–*33*). Spectroscopic studies and structural genomic surveys (*34*, *35*) suggest that this Atx1-like coordination chemistry is commonplace in the growing list of homologous bacterial Cu trafficking systems (*36*, *37*). Extensive studies of the *Enterococcus hirea* operon that contains the transporter CopB, chaperone CopZ, and repressor CopY frame several of these issues and have recently been reviewed elsewhere (*36*, *38*, *39*).

Mitochondrial Cu trafficking. An important destination for Cu in eukaryotes is the mitochondrial Cu and heme enzyme cytochrome oxidase (CO). The Cox17 protein, which is involved in the assembly of the active form of CO (*40*, *41*), binds Cu(I) through cysteine site chains but exhibits some important differences from other Cu-trafficking proteins. In some forms of the isolated protein, Cu is trigonally coordinated, either in a single hexanuclear cluster or in two trinuclear Cu(I) clusters, in a manner more similar to the metallothioneins and Cu(I) transcription factors than to the other Cu chaperones (*42*). Evaluation of this coordination site and the specific role of the Cox17 protein is ongoing, but recent studies suggest that Cox17 is not necessarily involved in conducting Cu to the mitochondria. Rather, it plays a key role, along with other proteins such as Sco1, in the assembly of the active CO in the intermembrane space of the mitochondria. The coordination chemistry of another assembly factor connected to Cu metabolism, yeast cox11, involves a dinuclear cluster of two Cu(I) ions bound by three cysteines each and may be a co-metallochaperone for the Cu_B site in CO (*43*).

Copper homeostasis in E. coli: *New Cu(I)-thioether chemistry*. A plethora of new proteins involved in Cu and Zn homeostasis have been found in *E. coli*, and some adopt new types of Cu(I)-thioether coordination chemistry in proteins involving methionine (Met)–rich motifs, as well as special use of the periplasm. A series of genes (Fig. 2) are induced in a stepwise fashion as the Cu concentration in the growth medium is increased (*44*–*47*). The first are CueO, a multi-Cu oxidase whose expression is controlled by CueR (*45*), and CopA, a transporter related to the Menkes disease protein, which pumps Cu from the cytoplasm into the periplasm (*46*–*49*). CueO and related proteins, such as PcoA, are proposed to convert Cu(I) to less toxic Cu(II) (*50*); however, their exact role is not yet established (*51*). If Cu concentrations continue to rise, the *CusCBFA* genes are induced by a two-component system (CusRS), which typically monitors stress in the cell envelope and is particularly effective in anaerobic Cu stress conditions (*46*).

If increasing Cu concentrations overwhelm these chromosomally encoded pathways, cells that harbor either the *pco* (*E. coli*) or *cop* (*Pseudomonas syringae*) Cu resistance plasmids can invoke one last line of defense. The *pco* operon includes the bacterial multicopperoxidase PcoA and its putative partner PcoC, both of which are exported to the periplasm (*52*). Recently published spectroscopic and crystallographic data for PcoC and nuclear magnetic resonance (NMR) studies of the closely related *P. syringae* protein, CopC, reveal a biologically unprecedented thioether ligation (*48*, *50*, *51*, *53*–*55*). PcoC exhibits a cuperodoxin fold that binds Cu(I) through two Met sulfur atoms and one nitrogen or oxygen ligand in a hydrophobic Met-rich loop that is exposed to solvent on the protein surface. Yet Cu(II) can be bound at a separate site in the same protein, where it coordinates water, as well as two histidine imidazole ligands and two other nitrogen or oxygen ligands. In the case of CopC, the coordination of Cu(II) and the overall fold are similar, and the latter two ligands are Asp side chains (*54*).

This chemistry of the Cu(I) center in PcoC is reminiscent of the low-coordination-number Atx1 site that is sterically protected but allows a partner protein to access the site via conformational changes and coordination of an incoming side chain (*22*). The crystal structure of the apo form of PcoC suggests a flexible environment that can, like Atx1, stabilize two- or three-coordinate geometry for the bound metal (*53*). These low-coordination-number environments are well poised for metal transfer chemistry. It is interesting that all of the Cu trafficking proteins shown in Fig. 2 are involved in the movement of Cu from the cytoplasm or the periplasm out of the cell, and no Cu uptake genes have been identified in *E. coli* to date.

Unusual coordination sites involved in zinc homeostasis. Zn trafficking proteins are also proving to be sources of unprecedented biological coordination sites (Fig. 3). Recently, the solution structure of an Atx1-like N-terminal domain of the bacterial Zn efflux P-type adenosine triphosphatase (ATPase) ZntA was reported (*56*). This domain contains a -GMDCXXC- motif (*20*), similar to the metal-binding motif in the Cu-trafficking proteins (*34*); however, it binds Zn(II) via an aspartate oxygen and a potential oxygen or nitrogen ligand (from solvent or buffer) in addition to the two cannonical cysteine S atoms (Fig. 3). This Zn environment had not previously been seen in any of the structurally characterized Zn(II) proteins (*57*). Exactly how metal ion selectivity in P-type ATPases such as ZntA is achieved despite

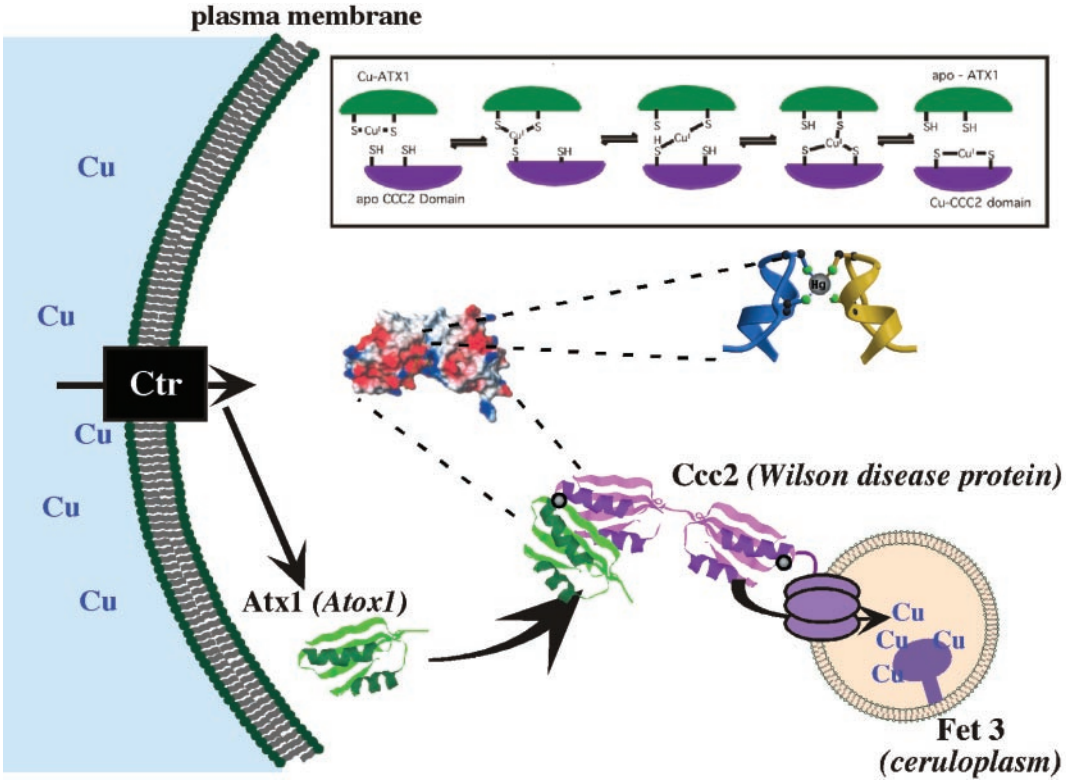

Fig. 1. Cu trafficking in *S. cerevisiae*. In the cytoplasm of yeast, Cu(I) can be conducted by the Atx1 copper chaperone, which delivers Cu to its target protein Ccc2. Ccc2 then brings Cu into a trans-Golgi compartment, where it is utilized by the multi-Cu oxidase Fet3. The names of the human homologs are shown in parentheses. A hypothetical mechanism for Cu(I) transfer involving the electrostatic docking of the partners is inset. The formation of a metal-bridged intermediate is supported by the crystal structure of Atox1, which is shown.

having -GMXCXXC- motifs (*20*) that are otherwise similar to the Cu(I)-specific transporters remains a controversial topic (*58*–*62*). The binding of aspartate to the Zn^{2+} ion as well as the two expected cysteines gives a complex with an overall charge of –1, which would prevent ionization of any water bound in the fourth site. This is one way that the transporter may suppress catalytic chemistry typical of hydrolytic Zn enzymes while leaving a labile coordination site open to the next protein that will acquire the Zn(II) ion. It is possible that metal selectivity is mediated via specific protein-protein interactions that distinguish between the various metal-binding loops (*62*).

Zn trafficking in eukaryotes is also being unraveled (*63*). The ZIP (Zrt-, Irt-like proteins) family of proteins is involved in Zn import into the cytoplasm. They are predicted to have eight transmembrane domains, and domains IV to VIII in these proteins are highly conserved. These transmembrane domains contain conserved histidines, which may be important in Zn binding. Likewise, the CDF family of transporters has been implicated in the transport of Zn out of the cytoplasm of yeast cells. They are predicted to have six transmembrane domains and a potential cytoplasmic metal-binding motif, -H(D/E)XHWXLTX$_8$H- (*20*), but neither the coordination chemistry nor the mechanism of the His-rich domains is known. These have recently been reviewed, as have the metallothionein transcription factors, which are of central importance in eukaryotic Zn homeostasis (*64*, *65*).

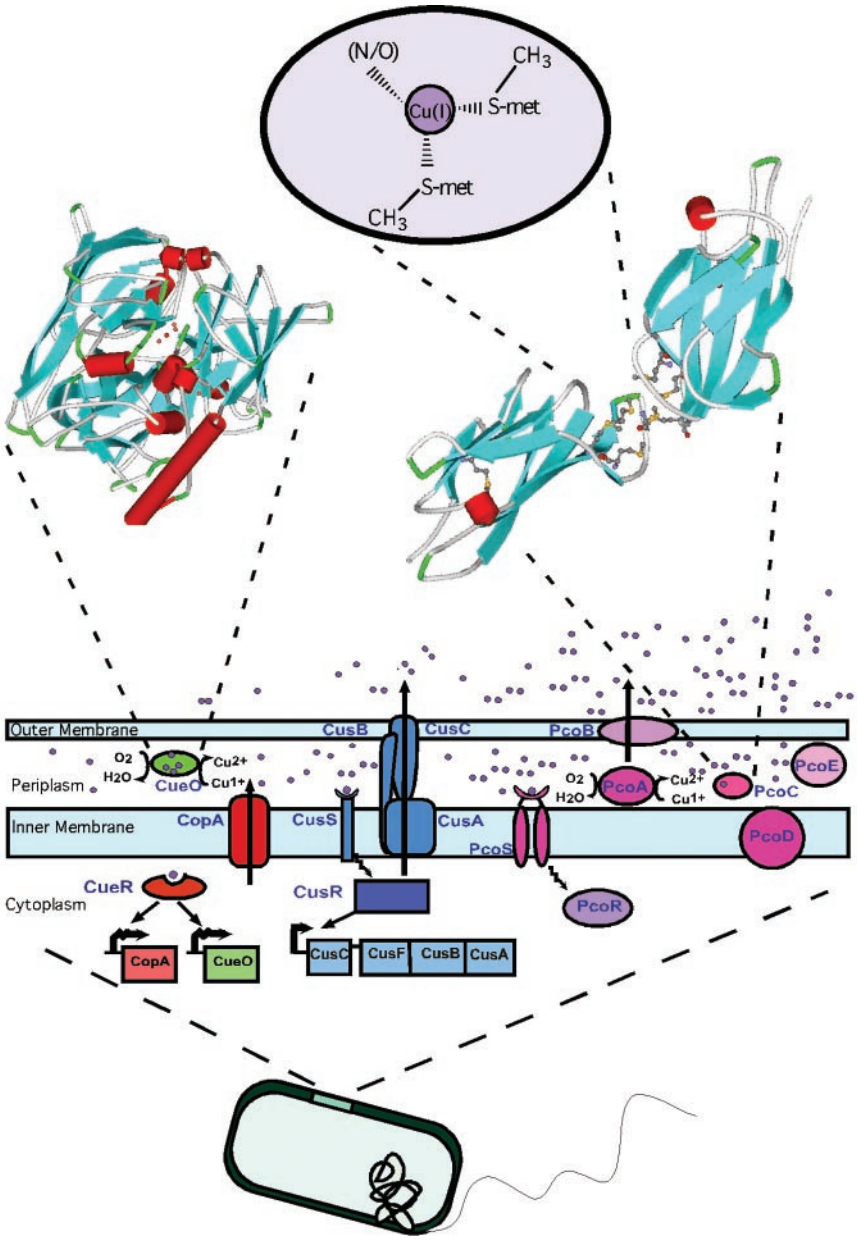

Fig. 2. Copper trafficking in the periplasm. The periplasm, a compartment of the cell envelope of Gram-negative bacteria, is proving to be an important site of Cu trafficking and utilization. Cellular Cu efflux is controlled in *E. coli* by the *cue*, *cus*, and *pco* operons, each of which is induced at different levels of Cu stress by separate metalloregulatory proteins. Recent structural insights for CueO and PcoC are highlighted. The cartoons of Cu ions (purple balls) represent various levels of total Cu content in the periplasm.

Emerging Metal-Trafficking Systems

Many other areas of metal ion trafficking are being elucidated, and we are beginning to understand how the intracellular speciation of other metal ions compares with that of Cu and Zn. A notable example is heme Fe insertion into cytochrome c. In the bacterial periplasm, a membrane-tethered protein known as CcmE, a putative heme chaperone, is required for this (*66*). The heme cofactor is thought to be covalently bound by CcmE in a transient manner (*67*); however, the transfer of heme from CcmE to Apo–cytochrome c is not readily reconstituted in vitro. The recent NMR structure of CcmE showed that there is no classic heme-binding site in the protein and, somewhat perplexingly, CcmE would not bind heme in vitro (*68*). This implies that the physiological function of CcmE may only be carried out in an assembly with other proteins required for cytochrome c maturation. This protein is more comparable to assembly factors than to diffusible metallochaperone proteins.

Another area of metal ion trafficking where a remarkable amount of progress is being made is in the study of a Ni homeostasis operon of *E. coli*, *nikABCDE*. Transcription of this gene cluster is regulated by the Ni-responsive repressor protein NikR (*69*). These studies find that in vivo, NikR is present in the cell at concentrations of at least 200 nM and that it binds Ni at picomolar levels, which seems to indicate that, as is the case for Cu and Zn, there is no free Ni in *E. coli*, although other interpretations have been suggested (*69*). Likewise, this protein also binds Ni with unique coordination chemistry (*70*). Studies of other areas of Ni trafficking are also quickly expanding this picture, including recent structural and biochemical studies of UreE, a putative Ni chaperone required for assembly of urease (*71*–*73*).

These new advances place the ongoing discussion about free Fe in the cytoplasm in a new light. It has been proposed that chemically free (or perhaps more precisely, biologically available) pools of Fe can be detected in *E. coli* by electron paramagnetic resonance spectroscopy in whole-cell extracts (*74*). If there were pools of free Fe ions in the cytoplasm, it would represent an anomaly in the trends discussed above. Complex transport and regulatory mechanisms for Fe are also an area of current study. Most human diseases of Fe transport and Fe overload are a result of mutations in Fe-export

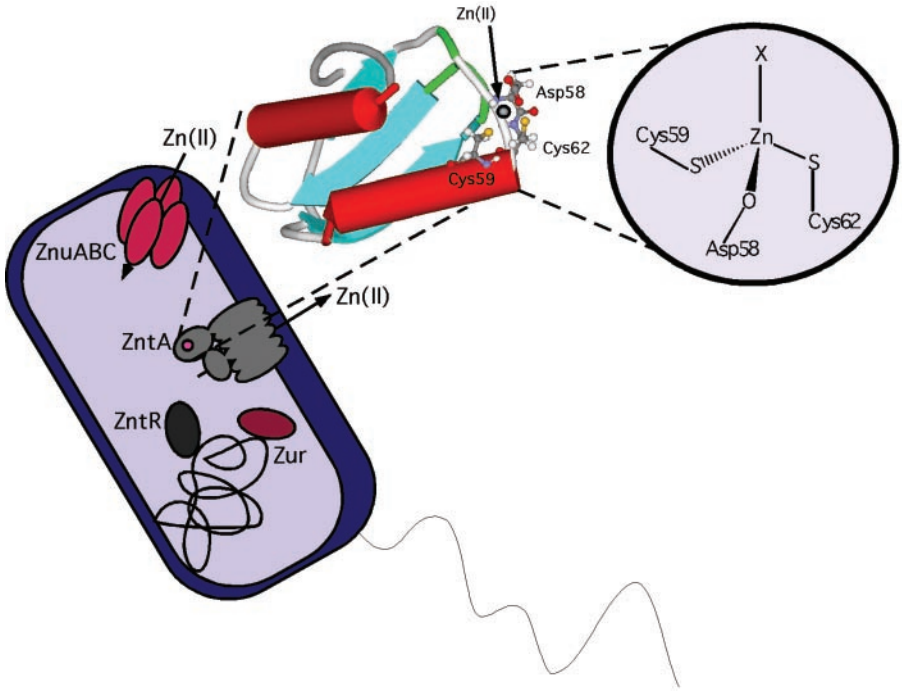

Fig. 3. Zinc homeostasis in *E. coli*. The trafficking of Zn(II) in the cell is controlled by the metal-sensitive regulatory proteins Zur and ZntR, which regulate the transcription of the pumps ZnuABC and ZntA, respectively. The recently determined structure of a metal-binding domain of ZntA is inset.

genes (*75*). Additionally, a suppressor screen has identified a gene in yeast, *ccc1*, the overexpression of which is able to prevent mitochondrial accumulation of Fe in the absence of the yeast frataxin homolog (*yfh1*) gene, causing it instead to accumulate in the vacuole (*76*, *77*). Other studies, such as those identifying Mn trafficking in yeast by Smf2p (*78*), illustrate how eukaryotic cells stringently control the distribution of metal ions between specialized compartments; however, little is known about the coordination chemistry of these proteins to date. Taken together, these studies suggest that additional intracellular Fe-, Zn-, and Mn-trafficking pathways are yet to be found. The discovery and study of such proteins and pathways are highly anticipated.

Prospects

Our understanding of inorganic physiology is still in its infancy; however, two themes are emerging: Metal transport across membranes and metal trafficking within the cytoplasm are accomplished with partners that share complementary folds, and these partner proteins exhibit active site chemistry unlike that seen in any of the hundreds of known metalloenzyme coordination environments. As more of the thermodynamics of the metal-trafficking proteins are elucidated, we see that there are often shallow thermodynamic gradients between the dissociation constants of partner proteins. This has opened the question of whether the kinetics of metal transfer and protein-protein interactions may control metal ion distribution among various chelators and functional active sites in the cell. Although it is clear that normal healthy cells concentrate significant amounts of many transition metals, controversies remain about whether cells operate metal-handling systems that guide and protect the uncommited metals at all times. Physical and chemical stresses and disease can disrupt the normal trafficking of metals, and, when unchecked, many of these metals are capable of catalyzing oxidative damage directly, inhibiting essential activities, disrupting signal transduction pathways, and disrupting the folding of nascent proteins. Such deleterious reactions are currently thought to be at the center of many inherited diseases of metal metabolism, including Menkes disease, Wilson disease, hematochromatosis, aceruloplasminemia, hypotransferrinemia, and others (*75*, *79*). Furthermore, a variety of neurodegenerative diseases such as Alzheimer's, Parkinson's, Creutzfeldt-Jakob, and neuronal damage caused by stroke and ischemia may be associated with pathological disruption of metal trafficking (*79*–*81*). It remains to be seen whether the excessive metal accumulation, aberrant protein folding, and/or extensive oxidative damage that are apparent at relevant sites in the brain and nervous system of afflicted individuals are the initiating events, protective responses, or end products of the specific disease.

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REVIEW

Boon and Bane of Metal Ions in Medicine

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In biological systems metal ions promote responses that range from deficiency to toxicity. Some, such as iron and zinc, have a known optimal intake range for normal, healthy individuals. Metal ions contained within well-designed molecules already constitute a great boon for the medicinal pharmacopoeia. However, whether essential or not, the threshold for toxicity can be very low. One of the challenges of designing metal-based drugs is to balance the potential toxicity of an active formulation with the substantial positive impact of these increasingly common therapeutic and diagnostic aids.

Not only are many metal ions essential nutrients, but many are also becoming increasingly prevalent components of diagnostic or therapeutic agents to study or treat a wide variety of diseases and metabolic disorders (*1*–*3*). The list of metal ions that qualify for essential status is a work in progress; it includes not only expected members such as zinc, copper, and manganese but also many formerly thought of only as poisons, such as selenium and molybdenum (*4*, *5*). Included in the "possibly essential" list are such unexpected candidates as arsenic, nickel, silicon, and vanadium (*5*–*7*). Although there is scant likelihood that these metal ions would turn out to be deficient in the general population, it is possible to show detrimental physiological effects under extreme conditions. Essentiality is no counterargument to toxicity, and vice versa (*8*). Therefore, appropriate intakes of metal-based therapeutics must be carefully defined.

A Question of Dose

Instead of the dichotomy of essential versus toxic, the concept is emerging of a dose-response continuum for each metal-containing complex, akin to other chemical compounds of biological or medicinal interest (*8*). This is not really a new concept; as the Swiss physician Paracelsus stated in the 16th century: "All substances are poisons: there is none which is not a poison. The right dose differentiates a poison and a remedy" (*9*).

For example, once recognized primarily as a toxic element, selenium is now incorporated in most multivitamin formulations and has known essential biochemical functions in selenoproteins and selenoenzymes in humans. Likewise, platinum, although not an essential element, is a critically important therapeutic adjuvant in cancer therapy, despite its well-known toxic potential (*10*). With the advent of cisplatin, a completely inorganic compound and a widely used chemotherapeutic, mortality from testicular cancer in young men has dropped from almost 100% to less than 10% in the past 25 years.

Current guidelines for appropriate intakes of trace metal ions are designed for "normal, healthy individuals"; however, this description may be inappropriate for large segments of the populace (*11*). For example, marginal deficiencies of essential minerals are implicated in the pathogenesis of various chronic diseases, including coronary heart disease, diabetes mellitus, nephropathy, and epilepsy. By contrast, an overload of a metal ion—the result of a genetic abnormality, a disease condition, environmental pollution, or even overzealous self-medication—can also lead to serious ill health. Pharmacologically beneficial intake ranges tend to be higher than recognized windows of optimal intake for nutrients, raising the specter of toxicity as a bane for those designing metal-based pharmaceuticals (*12*).

Not only the right dose, but also the right metal-ligand combination, is important. In a metal-containing compound, the ligand is often, but not always, an organic compound that binds the metal ion(s) and modifies the physical and chemical properties of the ion. One important feature of inorganic drug design is how the ligand affects bioavailability, where bioavailability is the amount of a dose that is functionally usable by an organism (*13*). Accurate measurement of bioavailability is challenging, however, because unlike organic pharmaceuticals, a disproportionately large fraction of a metal-based drug dose may be cleared rapidly from the systemic circulation.

Emerging Therapeutic Principles

Ten years ago Abrams and Murrer (*14*) considered the field of medicinal inorganic chemistry as one having "many important applications but few principles tying the field together." Now there are even more applications, and fortunately some unifying principles are emerging. First, dose makes the difference between boon or bane; second, the entire chemical entity, not just the metal ion, matters; third, a prerequisite for quantitative studies is the capacity for accurate measurement; and fourth, the bioavailability of a metal-containing compound determines its relative biochemical impact.

Whether from a genetic disorder or adventitious insult, treatments for both metal-ion overload and metal-ion deficiency rely on similar strategies. In Wilson's disease, in which copper accumulates to toxic levels in all body tissues, treatment involves chelation of the metal ion to facilitate its removal from the body (*15*). British anti-Lewisite, 2,3-dimercaptopropan-1-ol, was one of the first chelating agents used for this purpose; now penicillamine or trientine is preferred.

Iron-deficiency disorders (anemias) are among the most common diseases, affecting more than half a billion people worldwide. Treatment of iron-deficiency anemia ideally requires supplementing iron as a chelate, for

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