CHAPTER V

Transport and Storage of Metal Ions in Biology

Contents

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V.1. Introduction

Metal ions have unique chemical properties that allow them to play diverse roles in cellular biochemistry. Whether it is the capability of Cu to catalyze oxidation–reduction (redox) chemistry or of Zn to act as a Lewis acid in hydrolytic enzymes, these properties have rendered certain metal ions indispensable for living organisms. Some metals, like Zn, have become so biologically abundant that it is difficult to imagine a living organism being able to adapt to life without them. In addition to its enzymatic role, Zn is a structural cofactor for thousands of proteins that mediate protein–protein, protein–nucleic acid, and protein–lipid interactions. Perhaps the most commonly recognized of these motifs is the ubiquitous *zinc finger* domain first identified in transcription factor IIIA (TFIIIA). It has been estimated that as many as 1% of proteins encoded by the human genome contain zinc-binding domains of this type.

The essentiality of metal ions in biology is unquestionable. Yet, despite the relative abundance of inorganic minerals on Earth, many formidable hurdles impede the acquisition of metal ions by living organisms, thus making metal nutrient sufficiency a perpetual problem. The exigent nature of this need requires organisms to go to great lengths to scrounge enough nutrition from the environment to survive. To recognize this problem, one needs only the image of a deer rooting around in and drinking from a brackish mud seep in an attempt to acquire necessary salts, like sodium, that it cannot get from its diet of vegetation.

Of course, the acquisition of metal ions is important for humans as well. Many genetic diseases in humans are caused by mutations that alter metal ion metabolism

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(see http://www3.ncbi.nlm.nih.gov/disease/Transporters.html). Furthermore, as many as 2 billion people worldwide suffer from malnutrition owing to deficiencies of micronutrients such as iron (see http://www.who.int/nut/). These deficiencies are due in part to inadequate food supplies in many parts of the world. However, poor nutritional quality of some regional crop plants and nutrient-depleted soil also play major roles in this problem.

Mineral nutrient resources are frequently so limited that organisms often wage war to obtain them. For example, the success of microbial pathogens in causing human disease depends critically on their ability to obtain metal ions from the blood stream and host tissues. In fact, the concerted effort to limit the availability of metal ions to pathogens is a major part of human immune defense against infection. Invading microbes respond reciprocally by up-regulating their own metal ion scavenging mechanisms, precipitating a veritable tug-of-war. Both pathogen and host subsequently produce molecules known as cytolytic agents that punch holes in the cellular membranes of the opposing side allowing stockpiles of nutrients to leak out.

An understanding of metal ion transport is crucial for improving human health. However, a proper understanding of metal ion transport processes requires a comprehension of those factors that essentially limit the ability of living cells to distribute these ions to the right place at the right time. After all, the uniquely useful properties of metal ions are worthless unless they can be effectively harnessed by the cellular machinery.

What are the major obstacles preventing the acquisition of metal ions? First and foremost, one must look at the *bioavailability* of the elements. This term implies more than just the incidence of an element on Earth and includes its prevalence in environments where life is found. There may be plenty of nickel in the Earth's core, but life certainly does not persist there. This supply is of no use for an organism that needs nickel. Another aspect of bioavailability is the form in which an element is commonly found. Zinc sulfide minerals are certainly common enough in the biosphere, but in this form Zn is not very usable. Few organisms possess the ability to mobilize Zn from such a source. Lastly, inherent in the term bioavailability is the presence of chemical competitors that impede acquisition of a desired nutrient. Molybdenum may be the most abundant transition metal ion in the ocean and it certainly possesses many desirable chemical properties. However, it is mainly found as the oxyanion, molybdate, a species that, while highly soluble and amenable for uptake, is very similar to the much more common sulfate and phosphate oxyanions. Competition from these other oxyanions may seriously hinder the ability to transport molybdate into the cell.

Other chemical properties that influence uptake include the redox chemistry, hydrolysis, solubility, chelation of the free ion, and ligand exchange rates of metal ion chelates. As we will see, for the widely coveted nutrient, iron, the aqueous chemistry of a metal ion in the ecological niches where life persists governs the mechanism by which the nutrient is acquired.

Once an organism has found a bioavailable source of minerals another problem arises: Cellular membranes are effective permeability barriers that block passive diffusion of charged molecules such as metal ions. Thus an organism may find and ingest the correct amount of a mineral, but it still needs to absorb it. To deal with this obstacle, exquisitely efficient uptake mechanisms have evolved. These uptake systems use molecules embedded in cellular membranes, which we refer to generically as *transporters*, to facilitate the selective movement of inorganic ions across the barrier. Not only do these transporters facilitate the absorption of nutrients from the environment, they are also responsible for the proper distribution of metal ions within whole organisms and individual cells.

Another critical problem in the delivery of metal ions to their ultimate targets is transport within the cytoplasm or the space between membranes. The cytoplasm is

an environment filled with metal ion chelators such as soluble proteins, peptides (e.g., glutathione), and organic metabolites (e.g., citrate). These molecules may impede the ability of metalloproteins to acquire their metal cofactor by acting as competitive chelators. In the case of Cu, specific soluble transport proteins, called copper chaperones, have evolved to facilitate the safe transfer of Cu from the plasma membrane to various copper-containing proteins (see Section VIII.6). Similar proteins may exist for other metal ions.

The level of a particular metal ion available to an organism in its diet or environment can change drastically over time. Often, when food is plentiful, organisms are exposed to quantities of metal ions that exceed their requirement. The ability to store nutrients in a usable form during these times of plenty represents a sensible means of ensuring adequate nutrition during times of starvation. Indeed, storage mechanisms have evolved for a variety of nutrients. Storage mechanisms also provide a means by which excess metal ions can be detoxified. Overaccumulation can lead to a variety of toxic effects. As the intracellular level of a metal ion becomes excessive, the metal can inhibit critical processes, for example, by competing with other metal ions for enzyme active sites and other biologically important ligands. Excess Fe and Cu can also generate reactive oxygen species that damage DNA, lipids, and proteins.

Should the optimal range of metal ions be exceeded, various mechanisms limit the deleterious effects of the metal. These mechanisms include: *exclusion* of metals at the level of uptake, *extrusion* of accumulated metals out of the cytoplasm, and *detoxification* of metals by transformation into a harmless adduct or incorporation into an inert state. These mechanisms are also necessary to survive exposure to metal ions, such as Pb, that are not biologically essential, yet common enough to pose a serious biochemical threat.

Since metal ions can be both essential and toxic, a delicate balance, or *homeostasis,* must be maintained to keep the intracellular metal ion levels within an optimal range. Metal ion homeostasis is generally regulated tightly by sensors that govern the activity of transporters, storage molecules, and detoxifying enzymes (see Section XV.1). If metal ion deficiency is sensed, net ion uptake is increased and stored metals are mobilized. If metal ion excess is sensed, then net uptake is curtailed and storage capability is expanded. The two processes are intimately linked.

V.2. Metal Ion Bioavailability

The abundance of different metals in the Earth's crust can vary over several orders of magnitude (Chapter II; Table V.1). Iron, for example, is the fourth most abundant element in the Earth's crust and is 100-fold more abundant than Cu (geochemical considerations will be discussed in greater detail in Chapter II). Despite its abundance in terrestrial environments, the level of iron in some surface sea water can be as low as $50\,\mathrm{p}M$ ($5 \times 10^{-5}\,\mu M$). Could this extremely low level of iron restrict growth of aquatic organisms? This hypothesis was proven correct in experiments where iron supplementation at the surface of specific areas in the Pacific and Southern Oceans led to increased phytoplankton populations as measured by biomass levels. Thus, in many aquatic environments, as well as in some terrestrial environments, the abundance of metal ions can be growth limiting.

For mammalian cells, the source of metal ions is the blood plasma. Plasma levels of metal ions are controlled within a narrow range of concentrations by regulating absorption and/or excretion of metal ions from the body. Cells that obtain their metal ions from plasma accumulate most of these elements to much higher levels than the plasma itself (Table V.1). The observation that cells—mammalian and otherwise—must often acquire metal ions against a concentration gradient demonstrates the importance of efficient metal ion uptake systems. Notice in Table V.1 the

Table V.1.
Average Relative Abundance of Selected Elements in the Earth's Crust, Sea Water, Mammalian Blood Plasma, and in Mammalian Cells or Tissue

Element	Crust (ppm)	Sea Water (μM)	Blood Plasma (μM)	Cell/Tissue[a] (μM)
Ca	4×10^4	1×10^4	2×10^3	1×10^3
Cd	0.2	1×10^{-3}		
Co	25	2×10^{-5}	2.5×10^{-5}	
Cu	55	4×10^{-3}	8–24	~ 68
Fe	5×10^4	1×10^{-3}	22	0.001–10
K	3×10^4	1×10^4	4×10^3	1.5×10^5
Mg	2×10^4	5×10^4	500	9×10^3
Mn	950	5×10^{-4}	0.1	180
Mo	1.5	0.1		5×10^{-3}
Na	3×10^4	5×10^5	1×10^5	1×10^4
Ni	75	8×10^{-3}	0.04	2
V	135	0.03	0.07	0.5–30
W	1.5	5×10^{-3}		
Zn	70	0.01	17	180

[a] Approximate values based on total content rather than labile concentration.

contrasting behavior of Ca and Na. These elements are found in greater quantities in plasma than in cells. In these cases, it is equally difficult for cells to extrude the ions against a concentration gradient.

The chemical properties of metal ions can greatly affect their availability to organisms. The properties of Fe in particular have a major impact on its bioavailability. In general, the chemical properties of other metal ions are less of an obstacle to their uptake and simpler transport systems may be required. In a sense, the discussion of Fe chemistry will serve as an extreme example of the problems cells must overcome to obtain metal ions.

V.2.1. Iron: A Case Study

Approximately one-third of our planet's mass is iron (see Chapter VI). This great abundance would suggest that Fe is readily available to organisms for their use. However, this is far from the case because of iron's chemical properties. First, in aerobic environments, Fe is most prevalent in the Fe^{3+} oxidation state. Hydrated Fe^{3+} (ferric) ions are only stable in strongly acidic solutions. At higher pH values, hydrated ferric ions readily undergo hydrolysis to form iron hydroxide [ferrihydrite, $Fe(OH)_3$, Eq. 1]. The solubility product of iron hydroxide is 10^{-38} (Eq. 2). By using Eqs. 3 and 4 we can calculate the concentration of hydrated ferric ions at pH 7 to be $\sim 10^{-17}$ M. Clearly, the free ferric ion is all but insoluble at physiological pH in aqueous solutions and as such would be growth limiting.

$$[Fe(H_2O)_6]^{3+} \rightarrow Fe(OH)_3 + 3\ H^+ + 3\ H_2O \quad (1)$$

$$K_{sp} = [Fe^{3+}][OH^-]^3 \approx 10^{-38}\ M \quad (2)$$

$$[Fe^{3+}] = 10^{-38}/[OH^-]^3 \quad (3)$$

$$\text{At pH 7.0, } [Fe^{3+}] = 10^{-38}/(10^{-7})^3 = 10^{-17}\ M \quad (4)$$

Soluble Fe^{3+} can exist at much higher concentrations in an aqueous solution of neutral pH if it is bound to chelators such as citrate, ethylenediaminetetraacetic acid (EDTA), and so on. Such binding explains why there is radically more iron dissolved in the ocean than would be predicted by Eqs. 1–4 (see Table V.1). However, while these chelators allow for a higher level of soluble Fe^{3+}, they present additional problems to the process of cellular iron accumulation. Once bound, the metal ion may no longer be available for uptake. An important determinant in the bioavailability of chelated metal ions is their ligand exchange rates. The rate of ligand exchange for ferric ion chelates is generally very slow. Consider the following equation (Eq. 5):

$$[FeL_6]^{n+} + L' \rightarrow FeL_5L'^{n+} + L \tag{5}$$

where L and L′ are different ligands in an octahedral complex. The exchange rate for water bound to Fe^{3+} in such a reaction is on the order of $3 \times 10^3\ s^{-1}$ as compared to $3 \times 10^6\ s^{-1}$ for Fe^{2+} (ferrous iron). This reaction is representative of other ligand exchange rates, indicating that dissociation of Fe^{3+} from a chelator complex is much slower than for Fe^{2+}. Ligand exchange reactions are an important consideration in understanding transport of metal ions because the transport process can be thought of as principally a ligand-exchange reaction between the chelator and the transporter (Eq. 6).

$$[FeL_6]^{3+} + \text{Transporter} \rightarrow Fe^{3+}\text{-Transporter} + 6\ L \rightarrow \text{Transport} \tag{6}$$

Clearly, given its slow rate of ligand-exchange reactions, this reaction would probably not be an effective means of obtaining Fe^{3+}. Moreover, Fe is usually bound in multidentate chelate complexes such that there are additional entropic forces limiting the release of the metal (i.e., the chelate effect, see Tutorial II).

As described in more detail later in this chapter, given the solubility problems and slow ligand-exchange reactions of Fe^{3+}, a commonly used strategy of organisms to obtain iron is to reduce Fe^{3+} to Fe^{2+} prior to its mobilization. This strategy holds several advantages; Fe^{2+} is many orders of magnitude more soluble than Fe^{3+} at physiological pH values. The solubility product constant (K_{sp}) for $Fe^{2+}(OH)_2$ is 10^{-15}, thus $[Fe^{2+}]$ at pH 7 is 0.08 *M*. Moreover, Fe^{2+} is bound much less tightly by most organic acid chelators. As an example of this latter effect, the affinity of EDTA for Fe^{3+} is $\sim 10^8$-fold higher than its affinity for Fe^{2+}.

Another factor affecting the accumulation of metal ions in general, and Fe in particular, is the presence of high levels of other cations. For example, in humans, high levels of dietary Cu or Zn can inhibit Fe accumulation from the diet. This inhibition is probably due to competition of these different metal ions for a particular transporter (see Section V.3).

V.3. General Properties of Transport Systems

Before we discuss some of the specific systems responsible for metal ion transport, some general concepts of biological transport must be introduced. These concepts are critical to our understanding of the movement of solutes in and out of living cells and their intracellular compartments. Transport of an atom or molecule across a lipid bilayer can occur either by simple diffusion (i.e., nonmediated transport) or by transport mediated by molecules embedded in the membrane. Nonmediated transport occurs efficiently when the transported molecule is itself hydrophobic (like O_2) and can pass across the membrane unaided. Being charged entities, free metal ions require protein- or ionophore-mediated transport to cross a lipid bilayer.

Ionophores are a diverse class of organic molecules that increase the permeability of membranes to particular ions. Many ionophores are antibiotics produced by bacteria to inhibit the growth of other organisms by discharging the concentration gradients of important ions. One such example is gramicidin, an oligopeptide comprised of 15 alternating L- and D-amino acids. When inserted in the membrane of a Gram-positive bacterium, gramicidin forms a channel that is permeable to a variety of cations (Fig. V.1). Several non-peptidic ionophores function not as pores, but as carriers that dissipate ion gradients by physically shuttling ions across membranes. One such ionophore—the aromatic organic chelator pyrithione—is very effective at dissipating Zn^{2+} gradients. While ionophores are important for medical inorganic biochemistry, we focus here on protein-mediated transport because such processes are required for physiological metal ion transport in all cells.

The nomenclature of the protein-mediated transport field can be confusing, but there are essentially only three types of transport proteins: *channels*, *carriers*, and *pumps* (Fig. V.2). All of these proteins share the common feature of having several regions that span the membrane—designated transmembrane domains. These domains can be either α-helices or β-strands depending on the specific transporter. For our purposes, carriers are also referred to as *permeases*.

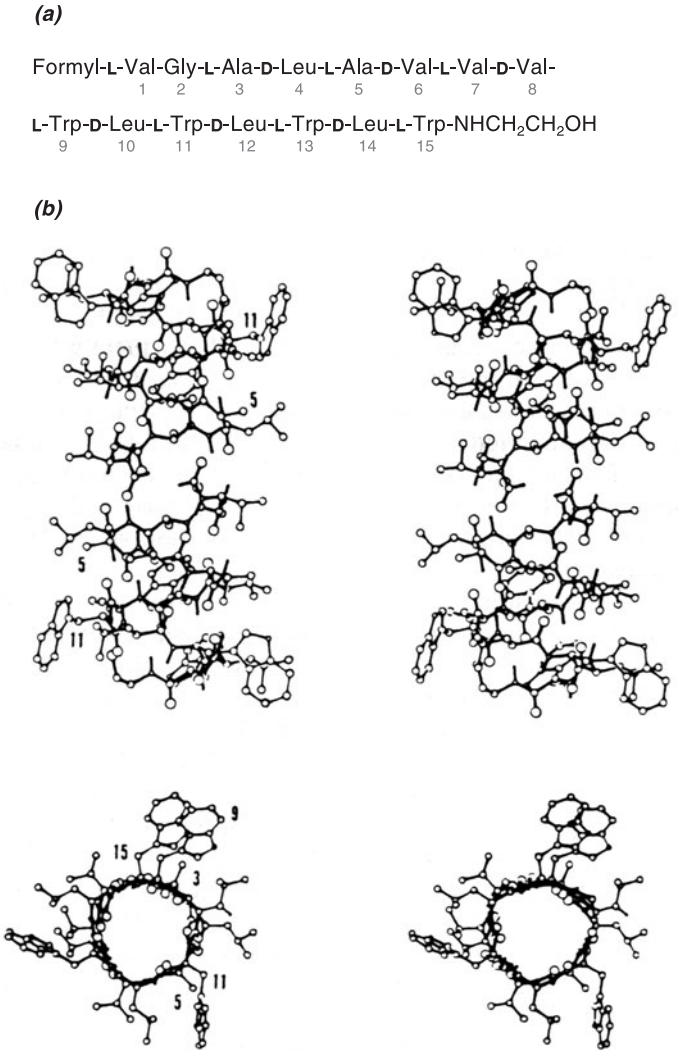

Fig. V.1.
The gramicidin channel.
(*a*) The chemical structure of gramicidin, a polypeptide with alternating L- and D-amino acids. (*b*) A stereoprojection model for the structure of the channel, based on physical measurements. The upper half of the figure shows a side view, the lower half an end-on view through the channel down the axis of the molecule. (Figure from Stein, **1990** with permission.)

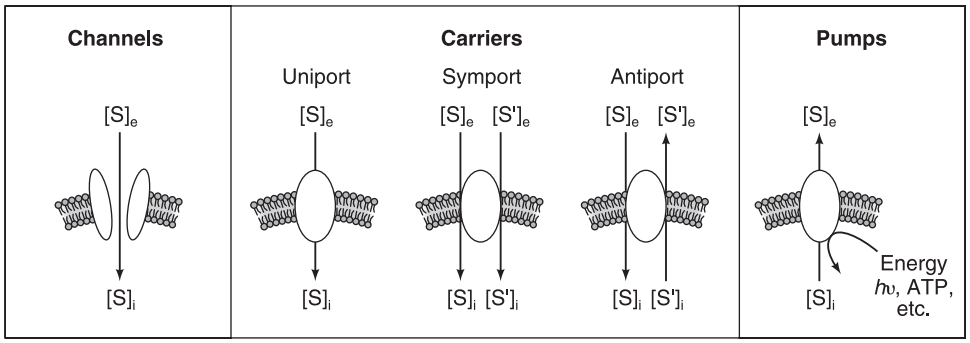

Fig. V.2.
Types of transport proteins. Three types of systems can mediate metal ion transport. Channel proteins utilize the chemical or electrical potential of the membrane to drive the transport of the substrate [S] through a pore in the membrane. The substrate is denoted by $[S]_e$ on the external side of the membrane and $[S]_i$ denotes substrate on the internal side of the membrane. Carrier proteins undergo a conformational change to facilitate movement of ions across membranes using the concentration gradient of [S] or the gradient of a cosubstrate [S′]. The cosubstrate can be transported in the same direction as the substrate (symport) or in the opposite direction (antiport). Pumps utilize energy, usually provided by adenosine triphosphate (ATP) hydrolysis (directly) to drive the transport of substrate ($h\nu$ = energy).

One crucial aspect of a transport system is the force that determines the direction of substrate movement. For proteins that simply facilitate the diffusion of cations, the concentration gradient or difference in *chemical potential* of the substrate is the major *driving force* such that the substrate will diffuse only in the direction from its high to its low concentration. For charged substrates such as metal ions, the cell membrane's *electrical potential* can also be an important factor. Membrane electrical potentials in living cells can be measured using microelectrodes and often range from -50 to -100 mV (inside negative). This electrical potential will favor uptake of cations and oppose their efflux from the cell.

V.3.1. Channels

Channels are proteins that form *pores* in the membrane that allow the movement of substrate across the membrane by diffusion. These pores can be gated such that they only open in response to signals such as changes in membrane electrical potential or ligand binding. In general, channels allow rapid transport of large quantities of substrate across membranes, thus dissipating a concentration gradient. An example of a channel protein is the ligand-gated *N*-methyl-D-aspartate (NMDA) receptor on the postsynaptic membrane of neuronal cells. This protein senses glutamate, an excitatory neurotransmitter, and responds by opening a Ca^{2+}-permeable channel that allows rapid influx of Ca^{2+} from the synaptic cleft, thereby transmitting a signal from one neuron to another.

V.3.2. Carriers

Carriers bind their substrate(s) on one side of a membrane, undergo a conformational change, and then release the substrate on the opposite side of the membrane. Some carrier proteins are *uniporters*, that is, proteins that transport only one type of substrate atom or molecule. Uniporters carry out a process known as *facilitated diffusion* and generally only allow transport along the concentration gradient. An example of such a system is the glucose transporter on the plasma membrane of human erythrocytes. A site on the external side of the membrane has a high affinity for glucose. Upon binding glucose, the conformation of the carrier protein changes in such a way that the site moves to the internal side of the membrane, if the concentration of glucose is low on that side where glucose is released. For example, there may be a high glucose flux through the glycolytic pathway due to a high demand for ATP and a consequent rapid rate of glucose oxidation.

Alternatively, carriers can be *cotransporters* that transport two or more substrates simultaneously. Cotransport of different substrates by a single transporter can occur in the same direction, called *symport*, or in opposite directions called *antiport*. As will be discussed in Section V.4, the Dmt1 transporter is responsible for the uptake of Fe

into mammalian cells. This transporter is an Fe^{2+}–H^+ symporter that uses a concentration gradient of protons and the electrical potential of the membrane to drive the uptake of Fe^{2+}. Because the driving force for transport of one substrate can be provided by the transport of the other, cotransporters allow for the accumulation of ions against a concentration gradient and are examples of *secondary active transport*.

V.3.3. Pumps

Finally, *pumps* use energy derived directly from the hydrolysis of ATP or other energy sources (e.g., light) to provide the energy for transport. Pumps are *primary active transport* systems and allow for accumulation of ions against a chemical gradient. An example of this type of primary active transport is the Ca^{2+}-ATPase on the membrane of the sarcoplasmic reticulum (SR). This transporter is responsible for the maintenance of low cytoplasmic calcium after muscle contraction by vesicular sequestration of cytoplasmic Ca^{2+}. The pump accomplishes this task by having two conformations (Fig. V.3). The E_1 conformation has two Ca^{2+}-binding sites on the cytoplasmic side of the membrane with a high affinity for Ca^{2+}. The Ca^{2+}- and ATP-dependent phosphorylation drives the conversion of the enzyme to the E_2 state. This conformational change has two main effects: (1) the Ca^{2+}-binding sites move to the luminal (inside) surface of the SR membrane, where (2) their relative affinity for Ca^{2+} decreases. Dissociation of Ca^{2+} followed by dephosphorylation prompt a return to the E_1 state.

Protein-mediated transport systems normally display a relationship between rates of substrate movement versus concentration that fits the Michaelis–Menten equation describing the rates of enzyme reactions (Eq. 7):

$$V = \frac{V_{max}[S]}{K_m + [S]} \tag{7}$$

where V is the rate of transport, [S] is the substrate concentration, V_{max} is the maximal rate of transport, and K_m is the concentration of substrate that gives a rate equal to one-half V_{max}. This relationship gives rise to a hyperbolic curve with saturation occurring at high substrate concentrations (Fig. V.4). This saturation effect is a characteristic of all protein-mediated transport systems. The parameter V_{max} values are

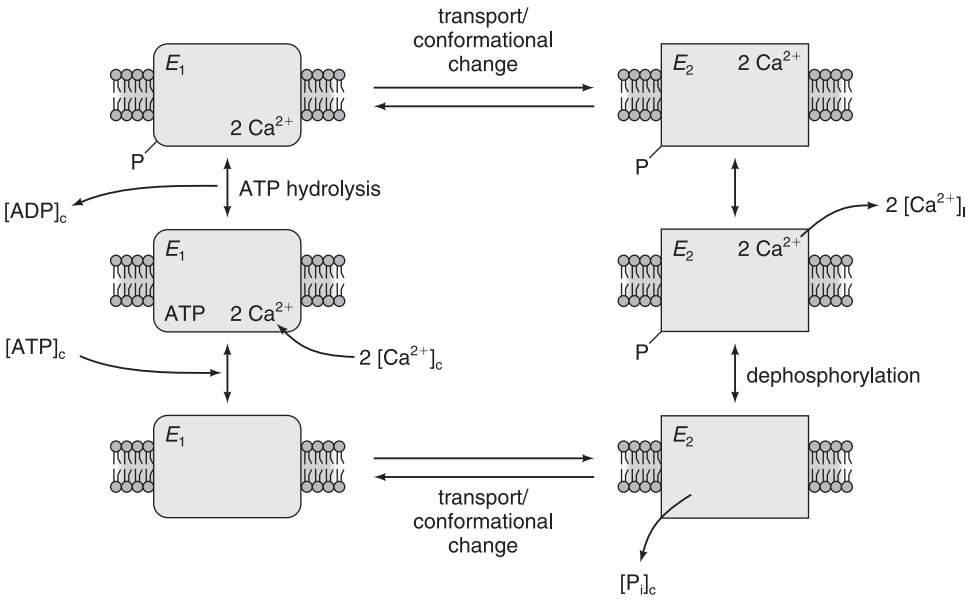

Fig. V.3.
Model of the Ca^{2+}-ATPase of the sarcoplasmic reticulum (SR). Low cytosolic calcium levels are maintained in the muscle cells via the Ca^{2+}-ATPase on the membrane of the SR. This protein transports Ca^{2+} into the SR using ATP hydrolysis as the driving force. This is achieved by a two-state mechanism (E_1 and E_2). In the E_1 state, the Ca^{2+}-binding sites are cytoplasmic and have a high affinity for Ca^{2+}. In the alternative conformational state, E_2, the Ca^{2+}-binding sites move to the lumenal side of the SR and have a much lower affinity for Ca^{2+}. Conversion from one state to the other is mediated by ATP hydrolysis and protein phosphorylation–dephosphorylation. Subscripts denote substrates on specific sides of the membrane; $[S]_c$ = cytoplasmic, $[S]_l$ = lumenal, P = phosphate, ADP = adenosine diphosphate.

indicative of either the prevalence of transporters on the membrane or the capacity of individual transporters, if their numbers can be measured. The K_m value of a transporter gives an estimate of the relative affinity of the protein for its substrate. The K_m values of metal ion transporters range from the low nanomolar (nM) range for carrier proteins to the millimolar (mM) range for most channel proteins.

Organisms often employ multiple transporters with widely varying affinities for the same substrate depending on its availability. For example, the yeast *S. cerevisiae* possesses 20 different hexose transporters (HXT), more than any other organism. Many of these HXTs function in the uptake of glucose from the medium and have widely varying K_m values for glucose transport. This allows the yeast to transport glucose (and therefore grow) at an optimal rate over a wide range of extracellular glucose concentrations (from μM to M).

Finally, what determines the substrate specificity of metal ion transporters? At this time, we know very little about how metal ion transporters distinguish between different metal ions. Presumably, this specificity is due to the same factors that affect metal ion binding by other metalloproteins (i.e., ionic radii, coordination geometries, and ligand preferences; see Chapter III and Tutorial II). Metal ion transporters have the added complexity of providing both specific and high-affinity metal binding along with a high degree of lability such that substrates are transported and efficiently

(a)

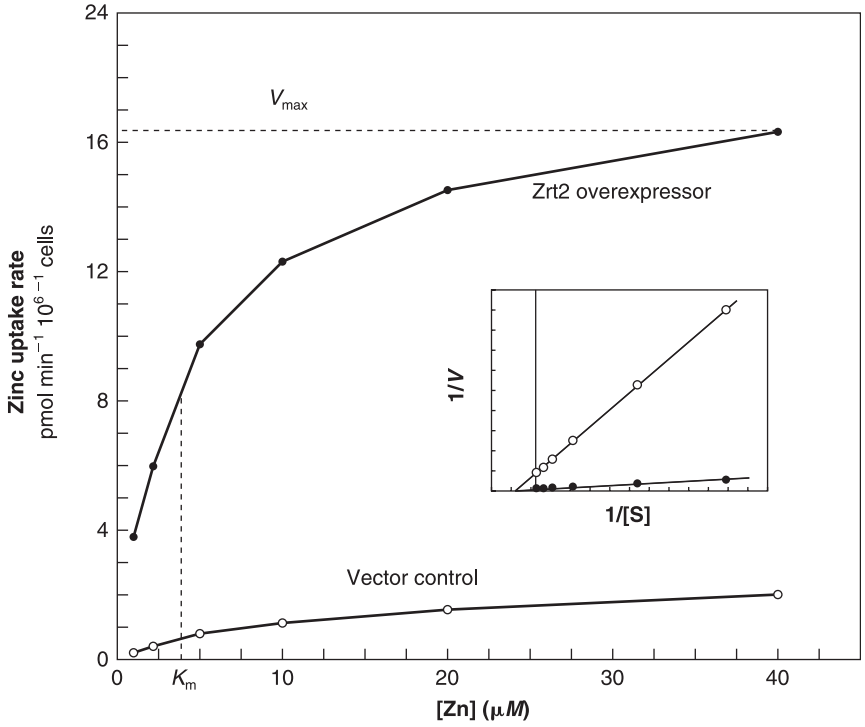

Fig. V.4.
Enzyme kinetics for the Zrt2 low-affinity zinc transporter from *Saccharomyces cerevisiae*. The Zrt2 transporter is overexpressed in a yeast strain that is deficient in high-affinity Zn uptake. The graph demonstrates that transporter activity is governed by classic Michaelis–Menten kinetics. Saturation of uptake, V_{max}, occurs at high concentrations of substrate; K_m, a measure of relative substrate affinity, is the substrate concentration at half-maximal uptake velocity. Inset shows a Lineweaver–Burk plot used to calculate kinetic parameters.

(b)

Strain	K_m	V_{max}
Zrt2 Overexpressor	3.6 ± 0.1	17 ± 2
Vector control	10 ± 1	2.0 ± 0.1

released following movement across the membrane. Elucidation of the way in which this combination of specificity, high affinity, and lability occurs represents an exciting area of research in bioinorganic chemistry.

V.4. Iron Illustrates the Problems of Metal Ion Transport

Iron is an essential nutrient to almost all organisms. But, as was discussed earlier, it can often be very scarce in the environment. While unusual in the natural world, one ingenious strategy used by some organisms to deal with the challenges posed by iron scarcity is simply to live without it. One organism that apparently does not require Fe is *Borrelia burgdorferi*, the bacterial pathogen that causes Lyme disease. This pathogen is an obligate parasite that survives Fe limitation in its human host by using manganese instead of iron in some metalloproteins and doing without other enzymes altogether. The lack of several of these important enzymes also explains why this bacterium is an obligate parasite and cannot grow outside of its host.

For those organisms that do require Fe for growth, several different pathways of Fe accumulation can be present in the same cell to ensure an adequate supply under a variety of conditions. For example, the baker's yeast *S. cerevisiae* has at least seven different Fe uptake systems (Fig. V.5). Gram-negative bacteria such as *Escherichia coli* have a similarly high number of uptake pathways available to them. Clearly, when it comes to obtaining Fe for growth, organisms leave little to chance.

Prior to transport, organisms must mobilize Fe. There are three general means by which mobilization is accomplished: *chelation*, *reduction*, and *acidification*. Each strategy serves to maintain Fe in a soluble form. Two general systems are then used to facilitate the transport of Fe across cellular membranes. Interestingly, mammalian Fe uptake systems combine all of these strategies and systems.

V.4.1. Chelation

Bacteria, fungi, and some plants use chelating agents called "siderophores" to obtain iron. Siderophores are small organic molecules that bind ferric iron with high affinity. Their structure, chemistry, and uptake mechanisms are discussed in Section VIII.3. These molecules are synthesized by bacteria, some plants, and some fungi and are secreted directly into the extracellular environment. Upon binding Fe, the extracellular iron–siderophore complex can be bound by receptors at the cell surface that also function as transporters for the complex. One such receptor–transporter is the FhuA protein of *E. coli*, discussed at length in Section VIII.3. Alternatively, the Fe^{3+} can be dissociated from the siderophore complex on the external surface and subsequently taken up by other transporters.

Siderophores have remarkably high affinity for iron due to their multidentate ligand character. Moreover, in addition to their high affinity for Fe^{3+}, these compounds also show extremely high specificity for ferric iron over other metals such that siderophores are very effective agents for specifically mobilizing extracellular iron, even when other metal ions might be present in much higher concentration.

Siderophores are effective devices in the competition between microorganisms for available Fe in the environment. Once bound by the siderophore, the Fe is no longer available to other microbes that are unable to use that particular iron–siderophore complex. Some organisms can actually take up Fe^{3+} complexes composed of siderophores produced by other organisms. One example is *S. cerevisiae*, an organism that does not produce its own siderophores, but has transport systems for a number of different iron–siderophore complexes (Fig. V.5). This clever strategy vividly illustrates the cut-throat world of microbial competition for scarce resources.

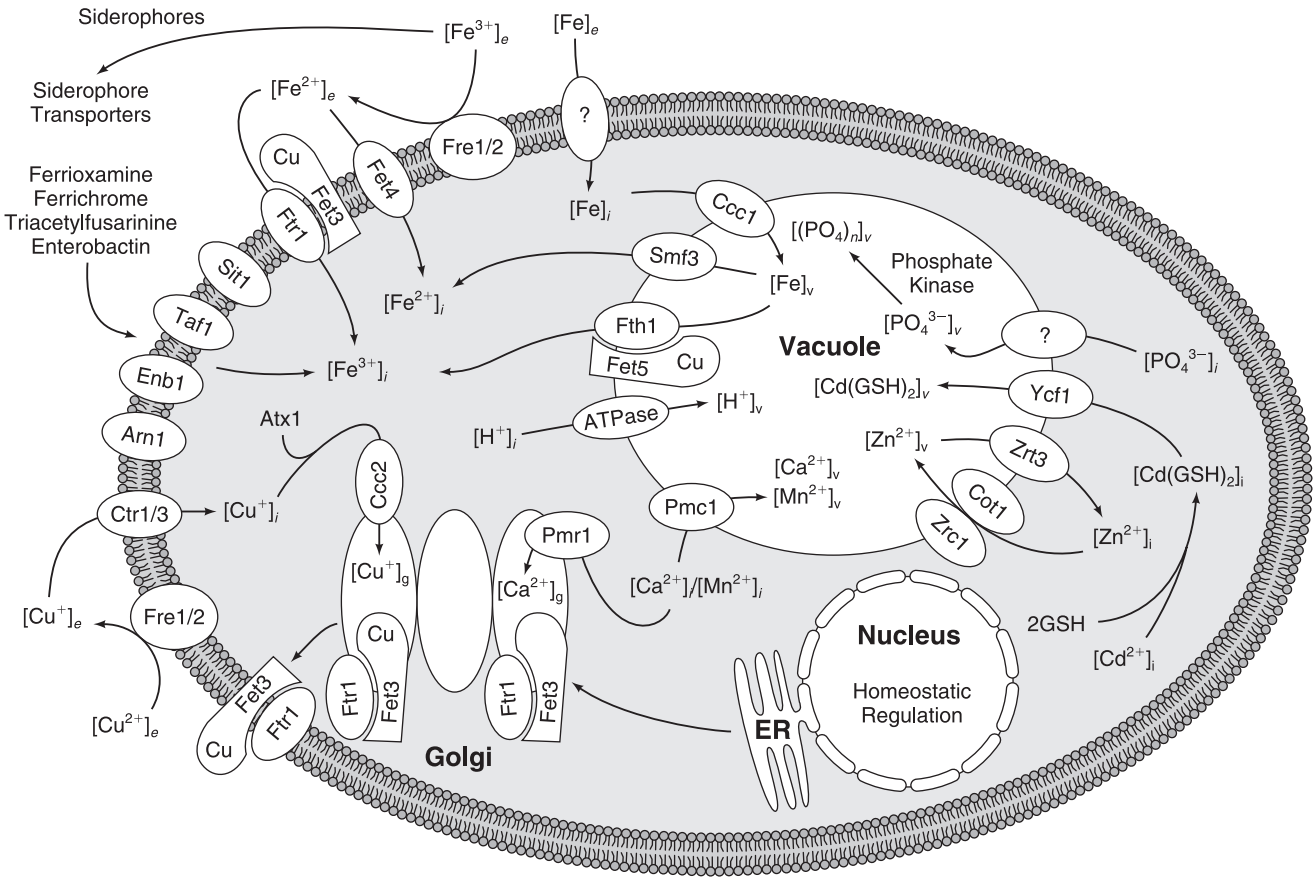

Fig. V.5.
Overview of Fe and Cu uptake systems and vacuolar cation storage in *S. cerevisiae*. Yeast possess at least seven different systems for acquiring Fe from the external milieu. Strains that lack all known Fe uptake systems are still viable suggesting that other routes of entry for iron are yet to be identified. The Fet3/Ftr1 high-affinity Fe uptake system requires Cu as a cofactor that is supplied by the action of Ctr1, Ctr3, Atx1, and Ccc2. Ion sensors in the nucleus and elsewhere regulate the activity of these systems and maintain ion homeostasis. The yeast vacuole is vital for metal ion homeostasis. Via its ability to accumulate large amounts of metals, the vacuole is critical for resistance to excess quantities of metal ions like Zn, Cu, Fe, Mg, Ca, and Mn. Vacuolar acidification and polyphosphate synthesis play crucial roles in this capacity. Mechanisms for mobilization of Fe and Zn from this storage pool are beginning to be understood (see text for details). Subscripts denote substrates in specific subcellular compartments; $[S]_e$ = external, $[S]_i$ = internal, $[S]_v$ = vacuolar, and $[S]_g$ = Golgi; GSH = glutathione.

V.4.2. Reduction

A second strategy of Fe uptake is to reduce extracellular Fe^{3+} to Fe^{2+} prior to uptake. This reduction dramatically increases the solubility of particulate Fe and can even liberate Fe from ores such as magnetite. The Fe^{2+} product is then transported into the cell by Fe^{2+}-specific transporters. This mechanism of uptake, typified by the major pathways of iron accumulation in *S. cerevisiae* (Fig. V.5), is common among other fungi, most plants, and mammals. The benefits of this strategy should be clear from the chemistry of iron. As discussed, Fe^{2+} is significantly more soluble, more kinetically labile, and binds with lower affinity to most chelators compared with Fe^{3+}. Thus, reduction increases the bioavailability of extracellular iron. In an atmosphere that has a significant partial pressure of dioxygen (pO_2), the newly generated Fe^{2+} oxidizes readily. Therefore, it is necessary to reduce the Fe close to the site of transport or in excess amounts. However, as we discuss later, many environments

are anaerobic or microaerobic and organisms that live under these conditions do not necessarily encounter the Fe solubility problems created by aerobiosis.

Reduction of Fe^{3+} is mediated by a class of enzymes found in the plasma membrane called ferrireductases (or ferric-chelate reductases). These reductases are often cytochromes that transfer electrons donated by intracellular reductants [e.g., reduced nicotinamide adenine dinucleotide phosphate (NADPH)] across the plasma membrane of the cell. The available evidence suggests that two heme groups embedded in the transmembrane domains of the reductases are responsible for the transfer of electrons across the plasma membrane.

For ferrireductases to be useful in iron accumulation, they must be capable of reducing Fe^{3+} bound to a variety of different chelators that are found in the extracellular environment. This finding is clearly true for many such enzymes. In *S. cerevisiae,* for example, the Fre1 and Fre2 ferrireductases are capable of reducing Fe bound to chelators as structurally diverse as citrate, nitrilotriacetic acid, EDTA, and the siderophore ferrioxamine. Since *S. cerevisiae* possesses seven putative ferric reductase genes, it is possible that some of them have evolved to function in the uptake of Fe bound to specific chelators or siderophores. Obviously, the ability to reduce extracellular microbial iron–siderophore complexes must be of great utility to this yeast when it is growing in the wild and competing with other microbes for what is often a limiting amount of iron.

A reductive mechanism of Fe absorption is also important for the uptake of nonheme iron in human diets. It has been shown that cell-surface ferrireductases line the cells of the mammalian small intestine and reduce dietary Fe^{3+} prior to its uptake by Fe^{2+}-specific transporters.

V.4.3. Acidification

The third strategy for solubilizing Fe^{3+}, often used in conjunction with reduction-dependent pathways, is acidification of the extracellular environment. For example, iron deficiency in plants causes an increased extrusion of protons from the roots into the soil. Activation of plasma membrane H^+-pumping ATPases in response to iron deficiency is responsible for this acidification. The rate of proton release can be quite fast, reducing the pH of the surrounding soil to values of 3 or even lower. This lowered pH serves to increase the soluble concentration of Fe^{3+} by inhibiting formation of hydrolysis products. For example, at pH 3, the soluble maximum concentration of free Fe^{3+} is $10\,\mu M$ (see Eq. 3). This level of iron is sufficient to sustain cell growth. The lowered pH also stabilizes ferrous iron relative to ferric ion, thus disfavoring reoxidation by O_2. Moreover, the increased $[H^+]$ competitively inhibits binding of both Fe^{3+} and Fe^{2+} by extracellular chelators.

V.4.4. Iron Transport Systems: Fe^{2+} Transporters

The molecular identification of Fe^{2+} transport systems began with the cloning of the *FeoB* gene of *E. coli*. The FeoB gene encodes an inner-membrane protein that transports Fe^{2+} into the cytoplasm of the cell. Among eukaryotes, several Fe^{2+} transporters have now been identified. In *S. cerevisiae*, the Fet4 protein is responsible for Fe^{2+} transport (Fig. V.5). In plants, the Irt1 protein mediates uptake of Fe^{2+} from the soil into the roots, while in mammals, the Dmt1 transporter mediates uptake of Fe^{2+} in the intestinal lumen into the enterocytes lining the small intestine. In each case, these transporters are integral membrane proteins with multiple transmembrane domains that transport Fe^{2+} directly across the membrane.

All Fe^{2+} transporters described so far function in environments that have very low oxygen tension or are anaerobic, for example, the intestine (FeoB, Dmt1) or the rhizosphere (Irt1). Under oxygen-poor conditions, ferrous iron will predominate and it

is energetically more favorable to simply transport this species. Yeast are also capable of growing anaerobically. It is no surprise then that transcription of the gene that encodes the Fet4 ferrous iron transporter is induced 1000-fold by a shift from aerobic to anaerobic growth.

V.4.5. Iron Transport Systems: Ferrous Oxidase-Permease Linked Transport

Other Fe^{2+} transport systems use an ingenious mechanism of ion transport that takes advantage of the redox chemistry of Fe to drive the transport process. In *S. cerevisiae*, when iron is abundant in the medium, the Fet4 transporter is responsible for iron uptake. This protein has a relatively low affinity for its substrate with a K_m of 600-n*M* free Fe^{2+}. When iron becomes limiting, a second system is induced that has a 200-fold higher affinity for iron.

One of the surprising observations regarding this high-affinity Fe uptake system was its dependence on copper: Copper-deficient cells are also Fe deficient because of a defect in the high-affinity Fe^{2+} uptake system. This mystery was solved by the molecular and biochemical characterization of the protein subunits that make up the high-affinity transporters, Ftr1 and Fet3. The Ftr1 transporter is an integral membrane protein with multiple transmembrane domains that probably serves as the iron transporter. The Fet3 transporter is also an integral plasma membrane protein with a single transmembrane domain at its carboxy-terminus. The amino-terminus of the protein is remarkably similar to a family of enzymes known as multicopper oxidases. The properties and mechanism of multicopper oxidases will be discussed in greater detail in Section XII.7. These proteins share the ability to catalyze four single-electron oxidations of substrate with the concomitant four-electron reduction of O_2 to H_2O. In the case of oxidase-permease based Fe uptake systems, the substrate is Fe^{2+}. In the yeast system, the Fe^{2+} produced by ferrireductases (or present in the environment through the action of extracellular reductants) is oxidized to Fe^{3+} by the Fet3 multicopper oxidase on the external surface of the cell. The Fe^{3+} product is transferred directly from Fet3 to an Fe^{3+}-binding site on the Ftr1 permease. Then, following a conformational change, the Fe is passed across the membrane into the cell.

V.4.6. Fe^{2+} Transport versus Oxidase-Permease Mediated Transport: A Comparison of Strategies

A confusing aspect of oxidase-permease mediated iron transport systems is understanding why cells go to the energy-demanding trouble of reducing Fe^{3+} to Fe^{2+} and then reoxidizing it back to Fe^{3+} while it is still on the extracellular surface of the plasma membrane. One possible explanation for the paradox is that extracellular reoxidation may confer greater substrate selectivity on the high-affinity uptake system. To be transported by this system, the Fe substrate must go through several steps (i.e., binding to the oxidase, oxidation, transfer to the permease, and transport), each with some degree of specificity for Fe^{2+} or Fe^{3+}. The combined effect of this multistep pathway is complete specificity for iron over other metal ions. This specificity is clearly manifest in the yeast high-affinity system, which cannot transport any metal ions other than iron.

In contrast, all of the simpler Fe^{2+} transporters studied to date, including Fet4, Irt1, and Dmt1, can transport several metal ions other than Fe^{2+} including Zn^{2+}, Mn^{2+}, and Cd^{2+}. Apparently, the means by which the Fe^{2+} transporters identify and transport Fe cannot completely distinguish between Fe^{2+} and many other metal ions as transport substrates. This fact is important, given that other metal ions are usually found in much greater concentrations than iron and as such will be

efficient competitors for transport. This promiscuity definitely poses a problem for iron-deficient plants, which upregulate the iron transporter, Irt1, to scavenge Fe from the soil. These plants also hyperaccumulate Zn^{2+}, Mn^{2+}, and Cd^{2+} in the process.

If the hypothesis is correct that ferrous-oxidase mediated transporters exist to confer specificity for Fe over other metal ions, then one must logically ask, why do Fe^{2+} transporters exist at all? One answer may be as simple as where the transporters are meant to function. Oxidase-permease based uptake depends on the availability of oxygen as a cosubstrate. In microaerobic or anaerobic environments such as those discussed above, the oxidase would not be functional. Thus, under these conditions, Fe^{2+} transporters may be the only reliable means to obtain iron, despite their apparent substrate promiscuity.

V.4.7. Mammalian Iron Transport: A Combination of Strategies

Uptake of dietary nonheme iron from the lumen of the mammalian intestine is mediated by an Fe^{2+} uptake system conceptually similar to that described above for low-affinity yeast iron uptake. Dietary iron that is reduced either by cell surface ferrireductases or by reductants (e.g., ascorbate) in the diet itself is taken up into cells called enterocytes that line the small intestine. The transporter responsible for this uptake is the previously described Dmt1 protein. Once inside the enterocyte cytoplasm, the iron is subsequently exported across the membrane of the enterocyte into the blood stream by ferroportin/IREG1. Before its release into the serum, the iron is oxidized by hephaestin, and then bound as Fe^{3+} by transferrin, an $\sim$700 amino acid serum protein. Transferrin binds Fe with high affinity ($K_d = 10^{-22}\ M$ at pH 7.0) and plays the principal role in delivering Fe^{3+} to the cells of most tissues in the body. Transferrin is discussed in greater detail in Section VIII.1.

Uptake of Fe from transferrin into other cell types combines all three of the strategies of iron mobilization described above (i.e., chelation, reduction, and acidification). Transferrin plays an analogous role to the siderophores used for Fe uptake by bacteria, fungi, and plants; by binding Fe^{3+} with high affinity, transferrin maintains the Fe in serum in a soluble form that can be used by cells.

Analogously to siderophores, iron-loaded transferrin is delivered to receptors on the surface of cells for uptake, where it undergoes a process called ''receptor-mediated endocytosis'' (discussed in Section VIII.1). Once inside the endocytic compartment, Fe^{3+} is released from the protein by acidification of the endosome, reduced by a ferrireductase, and transported into the cytoplasm by the same transporter, Dmt1, that was responsible for intestinal Fe uptake. Thus, in order for iron to reach its intended cellular targets in mammalian cells, a combination of all of the different iron solubilization and transport strategies must be used.

V.5. Transport of Metal Ions Other Than Iron

The chemistry of copper also poses many problems for its uptake and distribution. Like iron the solubility and stability of copper are highly dependent on environment, oxidation state, and chelators. Fortunately, Cu^{2+} is stable and soluble in an aerobic atmosphere. However, the divalent form of ionic Cu does not seem to be the preferred species for transport. In the case of yeast, the Ctr1 and Ctr3 high-affinity transporters mediate copper uptake. Uptake by these systems is similar to iron uptake in that Cu^{2+} is first reduced to Cu^{+} by plasma membrane cuprireductases. In fact, the same reductases that reduce Fe^{3+} are responsible for Cu^{2+} reduction (Fig. V.5). The Cu^{+} ion is then the substrate for Ctr1 and Ctr3. This system seems potentially inefficient, as free Cu^{+} undergoes rapid disproportionation to Cu^{2+} and Cu^{0}

in aqueous solution and readily oxidizes in air. However, despite this inherent instability, Cu^{+} has faster ligand exchange rates and is bound by many organic chelators, although with lesser affinity than Cu^{2+}. The Cu^{+} ion also has more stringent electronic requirements regarding the geometrical distribution and chemical properties of its preferred ligand set, so reduction of Cu^{2+} to Cu^{+} prior to transport is likely to allow for a more specific transport system. Thus, reduction of Cu^{2+} to Cu^{+} during copper uptake provides some of the same advantages as the reduction of Fe^{3+} does in iron uptake.

While many gene products responsible for the uptake and distribution of metal ions are known, the transport mechanisms are still poorly understood. Some hydrated metal ions, such as alkali metals (Na^{+}, K^{+}) and alkaline earth metals (Mg^{2+}, Ca^{2+}), exist only in a single oxidation state. These ions are also common, highly soluble, and form complexes with very fast exchange rates. Thus, transport of these ions is less complicated and probably proceeds in a straightforward manner. Zinc (Zn^{2+}) is very similar in that it has only one available oxidation state. However, Zn is much less abundant and is insoluble at higher pH values, so acidification followed by high-affinity transport represents an efficient means for its acquisition.

Other transition metals such as Mn, Co, and Ni can exist in multiple oxidation states, yet exploitation of their redox chemistry during transport is not suspected since for each ion the divalent species (Mn^{2+}, Co^{2+}, Ni^{2+}) is most stable in aqueous environments. Again, acidification is the primary means for mobilization as their solubilities and stabilities are enhanced at low pH values. Cobalt is unique in that it is often acquired as part of Vitamin B_{12} and as such may be transported in this complex. These metal ions are inserted into proteins, where their oxidation state may change. It is unclear which oxidation state is utilized during the intracellular transport and insertion processes.

Finally, transition metals like V, Mo, and W are most often found in oxygen-rich environments as oxyanions (VO_4^{3-}, MoO_4^{2-}, WO_4^{2-}). In alkaline conditions, these inorganic anions are structurally similar to phosphates and sulfates and are probably transported by proteins that resemble phosphate and sulfate permeases. However, acidic conditions tend to complicate their chemistry and promote formation of polyanionic species containing multiple metal ions or cationic species, depending on the concentration. Therefore, the transport of these metals as oxyanions may not be as simple as is commonly suspected.

Toxic metal ions, such as cadmium and silver, probably enter the cell via transporter proteins involved in taking up essential elements. For example, the Dmt1 Fe^{2+} transporter can also transport Cd^{2+} and Pb^{2+}. Thus, Dmt1 probably represents a significant entry point into the body for these toxic elements that can sometimes contaminate our diets.

V.6. Mechanisms of Metal Ion Storage and Resistance

The levels of essential metal ion nutrients available to an organism in its diet or surrounding environment can vary widely. Therefore, in addition to possessing efficient uptake mechanisms for metal ion accumulation, organisms also have the means to store essential metal ions when they are abundant for use at later times when those elements become scarce. These storage systems have the added benefit of allowing the accumulation of high intracellular levels of metal ions without the toxic consequences that such accumulation might otherwise entail. It should come as no surprise that many of the mechanisms involved in storing essential metal ion nutrients also play a role in detoxifying toxic, non-nutrient metals. Therefore, these two topics are considered together in this section.

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Cells employ two main strategies for metal ion storage that also serve to prevent toxicity due to overload of essential and non-nutrient metal ions. First, the metal ion may be bound by cytoplasmic proteins or macromolecules thereby keeping the level of free metal ions low. Certainly, the best understood systems of metal ion storage are the ferritin and metallothionein proteins (see Sections VIII.2 and VIII.4). Alternatively, metal ions can be transported into membrane-bound compartments within the cell (e.g., the plant and fungal vacuole), where the metal is less damaging. An additional strategy for detoxification of toxic metal ions, such as Cd^{2+}, is to pump them out of the cell so they will be diluted in the extracellular environment. Metal ion efflux does not just play a role in detoxification of harmful metals; it is also a major mechanism regulating levels of nutrient accumulation in mammals.

A recurring theme for metal ion storage and detoxification systems is that they are induced by metal ion exposure. This regulation links the storage and detoxification capacity of the cell to the level of exposure. As you will see in Chapter VIII, this control can be exerted at transcriptional, translational, or even post-translational levels.

V.6.1. Ferritin

Found in vertebrates, plants, some fungi, and bacteria, ferritin is the primary site of Fe storage in most organisms. The structure, chemistry, and biology of ferritin are discussed in greater detail in Section VIII.3. In brief, ferritin is a spherical molecule with an outer coat of protein and an inner core of hydrous ferric oxide $[Fe_2O_3(H_2O)_n]$. As many as 4500 atoms of Fe can be stored in a single ferritin molecule. Thus, when fully loaded with iron, soluble Fe concentrations as high as 0.25 M can be easily achieved *in vitro*. Comparing this value to the solubility of Fe^{3+} in solution at pH 7 (10^{-17} M) demonstrates just how useful this system is to the cell for storing iron in a usable form. Ferritin levels increase in cells treated with high levels of iron due to a post-transcriptional control mechanism that regulates translation of the messenger ribonucleic acid (mRNA) (Section VIII.2). However, while a great deal is known about the structure, metal-binding properties, and regulation of ferritin protein synthesis, we do not yet fully comprehend the mechanisms that control Fe loading during abundance and mobilization during scarcity.

V.6.2. Metallothionein

Another cytoplasmic metal-binding protein involved in metal ion storage and detoxification is metallothionein. Metallothioneins are small, cysteine-rich proteins that bind Zn^{2+}, Cd^{2+}, Cu^{2+}, and other metal ions by virtue of their Cys ligands. As many as seven Zn^{2+} or Cd^{2+} ions can be accommodated in the metallothionein structure. Widespread in Nature, metallothioneins are found in cyanobacteria, fungi, plants, insects, and vertebrates. A more detailed discussion of these proteins is found in Section VIII.4.

Metallothioneins bind metal ions with high affinity. For example, the stability constant for the Zn_7MT II complex is 3.2×10^{13} M^{-1}. Despite this high affinity of binding, at least some of the metal ions bound to metallothionein are kinetically very labile and can be readily donated to other ligands, such as Zn^{2+}-binding proteins, *in vitro*. Therefore, metallothioneins are thought to be an *in vivo* store of labile metal ions, particularly Zn^{2+}. Synthesis of some metallothionein isozymes is induced at the level of gene transcription by metal treatment. As with ferritin, this induction links the metal storage and detoxification capacity to the availability of the ion.

Mice lacking metallothionein show reproductive deficits during dietary zinc deficiency. These same mice are also more sensitive to the toxic effects of Cd. Thus, metallothioneins seem to play a dual role in Zn^{2+} storage and Cd^{2+} detoxification.

V.6.3. Other Intracellular Chelators

Some organisms use intracellular chelators in metal ion storage. In the fungus *Neurospora crassa*, the hydroxamate siderophore ferricrocin is used as an Fe storage pool in spores. Alternatively, small polypeptides called phytochelatins (PCs) can also play a role in metal ion storage and detoxification in plants and fungi. These PCs have the structure $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, where $n > 1$. While PCs are polypeptides, they are generated by enzymatic synthesis rather than by translation. The structure of phytochelatin indicates that it is related to glutathione ($n = 1$) and, indeed, glutathione is used as a precursor by the enzyme, phytochelatin synthase, that produces this compound (Fig. V.6).

As was the case with ferritin and metallothionein, the synthesis of PCs is also regulated by metal ions. This control occurs at a third level of regulation, post-translational control of enzyme activity. As depicted in Fig. V.6, a number of metal ions stimulate the activity of PC synthase *in vitro*. Synthesis continues until the metal ion is bound by the accumulated PC (or by adding a chelator such as EDTA) at which point the reaction stops. *In vivo* this mechanism provides an elegantly simple method to regulate PC synthesis in response to metal ion levels in the cell.

V.6.4. Intracellular Transport in Metal Ion Storage and/or Resistance

It is clear that macromolecules can act as internal stores for essential metals and as inert sinks for toxic metals. Metal ions can also be stored within membrane-bound organelles inside the cell, thereby eliminating their harmful effects in the cytoplasm. For example, the vacuole of fungi and plants appears to play a storage role for many metal ions including Fe^{2+}, Ca^{2+}, and Zn^{2+}. Several vacuolar functions have been shown to be essential for resistance to high levels of these same ions. One of these functions is the vacuolar H^+-ATPase, which is responsible for the acidification of the compartment. Another function is the accumulation of polyphosphate anions, which may provide a counterion to balance the accumulated positive charge as well as perhaps binding the metals in an inert form (Fig. V.5).

Other organelles seem to play a role in metal ion sequestration. The Ca^{2+} ion accumulates in mitochondria and secretory vesicles where it is stored for signaling purposes. Labile Zn^{2+} has been detected in membrane-bound organelles in mammalian cells and these may also play a storage or signaling function.

If these organelles (vacuole, secretory vesicle, mitochondria) are storing metal ions for later use, how are these metal ions mobilized? Recent advances in this field have come from the study of zinc and iron accumulation in the yeast vacuole. While two yeast proteins of overlapping function, Zrc1 and Cot1, have been shown to transport zinc into the vacuole, another protein, Zrt3, has been shown to be involved in the mobilization of this pool during zinc deficiency. In a similar fashion, iron is stored in the yeast vacuole via a protein called Ccc1. An oxidase-permease system comprised of the Fet5 and Fth1 proteins (homologous to Fet3 and Ftr1) has been postulated to mobilize stored iron pools. Another protein, called Smf3, is also suspected of

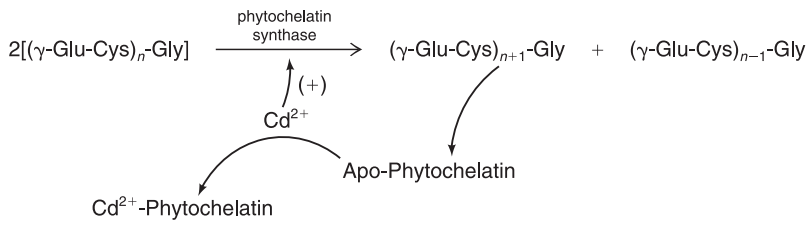

Fig. V.6.
Mechanism of phytochelatin synthase. Phytochelatins are synthesized from glutathione monomers. In response to heavy metals (e.g., Cd^{2+}), the enzyme phytochelatin synthase is activated via metal binding to thiol residues. The enzyme synthesizes phytochelatin by polymerizing γ-glutamylcysteine units (from glutathione or other phytochelatins). The newly synthesized phytochelatins then bind heavy metal ions, thus inhibiting the activity of phytochelatin synthase.

mobilizing vacuolar iron stores. Not surprisingly, the gene that encodes Smf3 is induced by anaerobiosis so that iron can still be exported under anoxic conditions when the Fet5/Fth1 oxidase-permease is nonfunctional. Mobilization of metals from cytosolic pools like ferritin and metallothionein are equally challenging issues, such that the exact nature and mobilization of these stored pools of metal ions remains an exciting line of research.

V.6.5. Exclusion

When it comes to the toxic effects of metal accumulation, *exclusion* is often one of the best strategies. Many cell types simply exclude the offending metal ion from the internal milieu by removing its routes of entry. One such method is the post-translational regulation of the activity or localization plasma membrane transporters. An example of this approach is the zinc-induced endocytosis and degradation of the high- and low-affinity zinc uptake systems in *S. cerevisiae*. Another elegant example of exclusion is the secretion of sulfide by yeasts in order to form insoluble extracellular complexes with metals like Cu and Cd.

V.6.6. Detoxification

Exclusion, however, is not always the best option for preventing metal toxicity. Sometimes, *detoxification* of a metal ion is more advantageous. One strategy for *detoxification* is to render metals harmless by internal chelation. Metallothionein and phytochelatin play this role for Cd^{2+}. A classic, yet certainly unusual example of metal ion detoxification is the *mer* operon in bacteria. This system involves the deliberate uptake of toxic Hg^{2+} ions and their conversion to volatile Hg^0 vapor thus rendering them diffusable and harmless to the organism. An ingenious use of this mercuric reductase system is in the process of phytoremediation. In principle, a plant that overexpresses a bacterial mercuric reductase could detoxify Hg^{2+} contaminated soil. This was indeed shown to be possible with the development of poplar trees that were transgenic for the mercuric-reductase gene. These transgenic plants volatilized Hg^0 at 10 times the rate of untransformed plants.

V.6.7. Extrusion

One of the simplest mechanisms to detoxify metals is simple *extrusion* from the cytoplasm. In *E. coli*, for example, excessive Zn accumulation activates transcription of a pump known as ZntA, a Zn^{2+} translocating P-type ATPase that expels excess Zn from the cell. Similar transporters are responsible for the efflux and detoxification of Cd, Cu, and Ag in a variety of bacterial species. *Extrusion* is not limited to expulsion from the cell entirely. Some resistance mechanisms transport toxic metal ions into storage compartments rendering the metals inert. Yeast compartmentalize excess Ca^{2+} and Mn^{2+} into both the vacuolar and Golgi compartments using the Pmc1 and Pmr1 ATPases, respectively. In an interesting combination of strategies, Cd^{2+} is detoxified first by conjugation to glutathione and then by transport into the vacuole by an ABC-type transporter called Ycf1.

V.7. Intracellular Metal Ion Transport and Trafficking

V.7.1. Trafficking

Metal ion transport into intracellular compartments for storage and detoxification requires that specific transporter proteins be present in the membranes of these organelles to facilitate this movement. Moreover, metal ions need to be trans-

ported into cellular organelles to function as cofactors of compartmentalized metal-dependent enzymes. For example, Fe, Cu, Mn, and Zn are required in the matrix of the mitochondrion. Iron is required in the matrix for the formation of heme and Fe–S clusters. Copper is required for the function of cytochrome *c* oxidase in the electron-transport chain. Manganese is a cofactor of the mitochondrial isozyme of superoxide dismutase, while Zn is required for the activity of proteins that proteolytically process matrix proteins and also for function of the mitochondrial RNA polymerase. For each of these metal ions, transporter proteins are required in the mitochondrial membranes to allow their movement into the organelle. Similarly, metal ions are required for various functions in the organelles of the secretory pathway, and transporters are needed to get them there as well.

In general, we know little about the transport mechanisms that are responsible for organellar metal ion trafficking. However, one excellent example of intracellular transport where the participants are well known is the transport of Cu into the secretory pathway. This transport is required for the activation of secreted or cell surface copper-dependent proteins such as lysyl oxidase, ceruloplasmin, ethylene receptors, and the yeast multicopper ferrous oxidase, Fet3. Moreover, in the mammalian liver, regulated Cu transport into the secretory pathway is the major site of control for Cu levels in the body. Excess Cu is transported into the bile caniculi for excretion.

The transport of Cu^{+} into the secretory pathway, specifically organelles of the Golgi apparatus, is mediated by eukaryotic members of the P-type ATPase transporter family (i.e., the same type of proteins responsible for metal ion efflux in bacteria). These proteins are discussed in Section VIII.5. A distinguishing feature of these proteins is the presence of a structural domain containing the heavy metal-binding motif MXCXXC. In yeast, the copper transporting ATPase of the secretory pathway is known as Ccc2 (Fig. V.5). In mammals, closely related transporters are called Mnk and WD because mutations in these genes cause the disorders of copper homeostasis known as Menkes and Wilson's disease.

V.7.2. Metallochaperones

Recent advances in our understanding of the intracellular trafficking of copper highlight the potency of research at the interface of biology and chemistry. Given the potential toxicity of Cu and its tendency to bind to adventitious (undesirable) sites, it is unlikely that the free ion is normally found at appreciable levels in the cytoplasm. In fact, it was recently demonstrated that there is no detectable free Cu in the cytoplasm. This surprisingly low level of Cu available for newly synthesized Cu enzymes presents a remarkable challenge to the cell, that is, How can a desired ligand compete with all the other potential copper-binding sites in the cell for miniscule amounts of available copper? We now know that the cell uses specific targeting proteins, called metallochaperones, to deliver Cu to intracellular targets. These proteins are discussed in Section VIII.6.

Copper metallochaperones are soluble, cytoplasmic copper-binding proteins. The proteins bind Cu after it enters the cell and deliver it to their corresponding recipient proteins. In yeast, for example, Atx1 is the copper metallochaperone that delivers Cu to the Ccc2 P-type ATPase (Fig. V.5) and Lys7 delivers copper to Cu/Zn superoxide dismutase. Most often, copper chaperones possess significant structural similarity to their target proteins and they often possess the heavy metal associated MXCXXC motif found in metal ion translocating ATPases. An important question is, Do metallochaperones exist for other metal ions? While we do not yet know the answer, it is entirely possible. Like Cu, Fe is very toxic to cells and homeostatic control mechanisms probably maintain very low levels of labile iron. Thus, the same challenges that lead to the evolution of copper chaperones also exist for iron.

V.8. Summary

This chapter has introduced many of the concepts underlying biological metal ion transport and storage. Subsequent chapters will consider, in much greater detail, several aspects presented briefly here. The chemistry of metal ions can create serious challenges to their accumulation by cells and this is especially true for iron. Organisms have evolved three general strategies to mobilize Fe from the extracellular environment: chelation, reduction, and acidification. The process of transferrin-dependent iron uptake in mammals is a good example of how all three of these strategies can be combined into a single iron transport pathway. Transport of mobilized metal ions depends heavily on the environmental conditions and the chemical properties of the metal. Many distinct pathways of Fe transport exist depending on external iron concentration, the presence of chelators, and the level of oxygen in the environment. Similar considerations may influence the uptake strategies for other metals as well. The uptake of metal ion nutrients is a highly regulated process governed by their intracellular availability.

Intracellular transporters are involved in moving metal ions in and out of organelles and across the plasma membrane. Metallochaperones shuttle their wards across the perilous cytoplasm full of potent metal chelators and sensitive components. Sensors regulate the activities of all of these proteins so that a balance, or homeostasis, is achieved and an optimal range of metal ion concentration is maintained.

When this balance is overwhelmed, or when an organism encounters a nonessential metal ion, storage and resistance mechanisms become necessary. Two basic strategies exist for metal ion storage. The first is binding by intracellular macromolecules and the second is their transport into intracellular organelles.

Three strategies exist whereby the toxic effects of metal ions can be abrogated. Metals can be excluded from the cell by regulation of transporter activity or by external sequestration. They can be detoxified within the cell by sequestration in macromolecules or by conversion to inert forms. Finally, they can be extruded from the cytoplasm either into storage compartments or into the extracellular space.

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