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Understanding How Cells Allocate Metals

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Abstract Life depends upon multiple metals. It is estimated that approximately one-third of all gene products require a metal for folding and/or catalysis. How does the correct metal locate to the correct protein? Provision of sufficient atoms of each of the

metals required by protein metal-binding sites is a challenge for cell biology. This is often especially true for iron, which is poorly soluble under aerobic conditions. Protein metal-binding sites follow universal affinity series. Under such a regime, exclusion of the wrong metals from metalloproteins is arguably an even greater challenge. High-fidelity homeostasis must match the number of some metal cations to the number of bonafide metal-binding sites. Selective protein–protein interactions also limit access of some atoms to the required subsets of proteins. Here we provide an overview of the contributions of metal sensors, metallochaperones, metal transporters and metal-storage proteins to the allocation of metals in cells. In this chapter an emphasis is placed on studies of the cell biology of metals in cyanobacteria.

1 The Irving–Williams Series and the Challenge for Homeostasis

The principles that govern the chemical speciation of metals in cells are clearly laid out in "*The Biological Chemistry of the Elements*" by Fraústo da Silva and Williams (2001) to which readers are directed. The underlying issue is that the metal sites of individual proteins are not sufficiently selective to solely bind the correct metal and exclude all others. Multiple partitioning events are required. Viewed as systems, cells monitor and modulate the number of atoms of each metal ion and the respective number of ligands. The homeostasis of different metal ions is also somehow integrated to avoid a surplus or deficiency of one element interfering with the speciation of another.

The Irving–Williams series was initially based upon empirical observations of the divalent transition metal-binding properties of model complexes and has been extended to include monovalent copper (Fraústo da Silva and Williams 2001). It specifies that the binding constants of proteins for essential metals will approximately follow a standard global order:

$$Mg^{2+} < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+}(Cu^{+}) > Zn^{2+} .$$

In a simple cell model, where all metal ions are equally available and in surplus to proteins, all metallo-proteins would become copper proteins. From this binding affinity series it follows that concepts of all nascent metalloproteins plucking the correct metal ions from free solution as they emerge from the ribosome, and of all transporters releasing metal ions to a cytosol that contains some concentration of the free ions, are grossly naive. The full suite of protein ligand chemistries, coordination geometries, and other physico-chemical properties, is inadequate for such a simplistic model, and therefore cells must have evolved to manage metal-protein speciation (Tottey et al. 2005).

From the perspective of bioinorganic chemistry, a series of pseudo-equilibria between the number of atoms of each metal ion and the number of ligands for each metal ion in any compartment can substantially resolve metal ion selectivity by becoming a function of relative affinity between dif-

ferent proteins in vivo, as opposed to absolute affinity. However, for molecular cell biology, this poses many more questions. What are the sensors? What are the regulatory circuits and how do they achieve the correct balance between metal ion number and ligand number? To date, some progress has been made towards understanding the control of the number of metal ions in cells. In bacterial systems, metal-responsive transcriptional regulators detect surplus or deficiency of specific metal ions and modulate production of metal-selective transporters and storage proteins and this is discussed in (Sect. 3). Several metal-responsive transcriptional regulators are also known in yeast (Rutherford and Bird 2004) but in higher eukaryotes, MTF-1 is the only well-described metal ion responsive transcriptional regulator (Lichtlen and Schaffner 2001). Much of the metalloregulation in eukaryotes acts at a post-transcriptional level, modulation of RNA stability or modulation of translation as occurs with IRP and iron-sulphur clusters (Pantopoulos 2004), or direct modulation of protein activity, localization or stability as occurs with several metal-transport proteins (Eisenstein 2000).

Control of the ligand number can sometimes be predominantly determined by modulation of a protein that functions to buffer surplus metal ion such as iron in ferritin (Hintze and Theil 2006), or zinc in bacterial metallothionein (Sect. 4.1). The ligand number can also to be adjusted by switching metabolic demand for a particular metal ion. For example, cyanobacteria, but not higher plants (Molina-Heredia et al. 2003; Weigel et al. 2003), undergo a metal-dependent switch between iron in cytochrome c_6 and copper in plastocyanin for photosynthetic electron transport but the control mechanism remains to be described (Raven et al. 1999) (discussed in Sect. 4.2). In the eukaryotic algae *Chlamydomonas reinhardtii*, an analogous response (Merchant 1998) involves the action of the Crr1 transcriptional regulator (Kropat et al. 2005). Crr1 also regulates tetrapyrrole biosynthesis in a copper dependent manner.

Kinetic factors influence the distribution of metal ions on proteins as reviewed by others (Finney and O'Halloran 2003; Fraústo da Silva and Williams 2001). Many metal-sites are buried within proteins and therefore exchange rates can be extremely slow. Once a metal ion is inserted into such a site it becomes trapped and unlikely to be replaced even with a metal ion that has greater affinity. Macromolecular assemblies and molecular crowding create chemically distinct microenvironments within the cytoplasm. Here we consider how the specificity of protein–protein interactions can influence the metallochaperone-mediated distribution of copper (Sect. 5), and where considerations of the relative affinities and allosteric mechanisms of metal-ion sensors are indicative of kinetic components to the control of selective metal-detection (Sect. 3).

It is worth noting that some proteins may only be occupied by the "correct" metal ion for some fraction of the time without becoming rate-limiting. In addition, while many enzymes show optimal activities with a specific metal

ion in vitro, it is probable that some metal ions that are suboptimal for catalysis are nonetheless adequate to sustain reaction rates required in vivo.

2
Cyanobacteria in the Study of Metals in Cells

"To begin with, for you to be here now trillions of drifting atoms had somehow to assemble in an intricate and curiously obliging manner to create you", "why atoms take this trouble is a bit of a puzzle" (Bryson 2003). Among these atoms are essential metal ions and much of the work to assemble them into larger molecules is, of course, done for you by photoautotrophs that synthesize organic molecules from inorganic atoms and energy derived from photosynthesis.

2.1
The Assimilation of Inorganic Atoms Into Organic Molecules

The chloroplasts of higher plants and algae arose via a single endosymbiotic event involving the ancestors of modern cyanobacteria approximately 1.26 billion years ago (Yoon et al. 2002). A typical sequenced cyanobacterial genome encodes in the region of 3000 proteins (3168 in *Synechocystis* PCC 6803, 2653 in *Synechococcus* PCC 7942) while *Anabaena* PCC 7942 has 5368 genes, which is consistent with the additional complexity associated with the differentiation of nitrogen fixing heterocysts under nitrogen-limiting conditions. Chloroplast genomes typically contain 110–120 predicted open reading frames. However, a substantial number of endosymbiont-derived genes have been retained within the plant genome; with up to 4500 open reading frames (18% of the total) in the *Arabidopsis thaliana* genome predicted to be derived from the cyanobacterial ancestor of plastids (Martin et al. 2002). One of the crucial activities of the products of these retained genes is the coupling of photosynthesis to the assimilation of inorganic elements. The inorganic cell biology of cyanobacteria therefore assumes a special significance.

By taking advantage of entire genome sequences it is possible to construct a most likely origin for the ancestral eukaryotic genome itself. It is widely accepted that the transcriptional machinery of eukaryotes is more akin to that of archaea than eubacteria. Genome-based phylogenies support the idea that the progenitor of eukaryotes was derived from a fusion of two diverse prokaryotic genomes, one of which was indeed related to archaeal prokaryotes (Rivera and Lake 2004). However, the other lies deep within an ancient photosynthetic clade that includes the cyanobacteria and α-proteobacteria, which are the groups that gave rise to chloroplasts and mitochondria.

The photosynthetic machinery has substantial demand for metal ions. It has been estimated that 80% of the iron content of plant leaves is con-

tained within chloroplasts (Terry 1983) and the cyanobacterium *Synechocystis* PCC 6803 requires ten times more iron than *Escherichia coli* (Keren et al. 2004). Photosystem I alone contains three separate iron-sulphur clusters of the Fe_4S_4-type and heme-iron is required for the associated cytochromes b_6f and *f*. The two photosystems and associated cytochromes require a total of 22 atoms of iron (Fig. 1). The oxygen evolving, water-splitting, complex of photosystem II requires four atoms of manganese. The manganese requirements of *Synechocystis* PCC 6803 are consequently 100-fold greater than a photosynthetic bacterium such as *Rhodobacter capsulatus*, which lacks the water-splitting enzyme (Keren et al. 2002).

Cyanobacteria have significance in relation to studies of metal-selection via insertion of ions into pre-formed chelating rings. These include tetrapyrroles of which cyanobacteria produce an atypically large repertoire that includes iron containing heme and siroheme, cobalt containing corrin for vitamin B_{12} plus magnesium containing chlorophyll. Higher plant chloroplasts do not produce vitamin B_{12} and contain no vitamin B_{12}-requiring enzymes. Many algae do have a requirement for vitamin B_{12} but possess no cobalamin biosynthetic machinery. It is proposed that these algae acquire vitamin B_{12} through a symbiotic relationship with bacteria (Croft et al. 2005). Association of the metal with the ring chelate is irreversible in vivo, other than via degradation of the organic molecule. The different rings are differently decorated and so metal-ion selection now becomes based upon proteins recognizing the

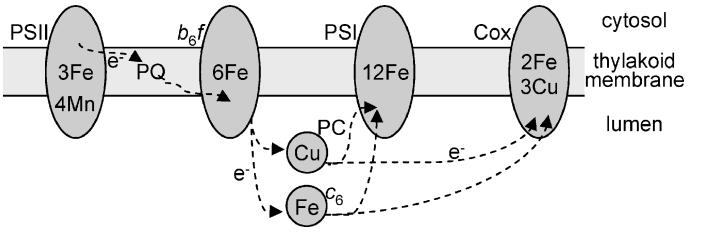

Fig. 1 *Synechocystis* PCC 6803 electron transfer in photosynthesis and respiration. During photosynthesis, electrons (e^-) are transferred (*dashed line*) from photosystem II (PSII) via the plastoquinone pool (PQ) to the b_6f complex, before being shuttled to photosystem I (PSI) via the soluble electron carriers plastocyanin (PC) or cytochrome c_6 (c_6). In some cyanobacteria, including *Synechocystis* PCC 6803, the soluble electron transporters can also donate their electrons to cytochrome oxidase (Cox). The location of the Mn, Fe, and Cu atoms are shown. PSII contains four Mn atoms in the water splitting complex, plus three Fe atoms consisting of two cytochromes and one haem Fe (Zouni et al. 2001). Cytochrome b_6f contains six Fe atoms comprising one $Fe_2 - S_2$ cluster plus four haems (Kurisu et al. 2003). The soluble electron transporters PC and c_6 contain one Cu and Fe atom, respectively, while PSI contains 12 Fe atoms in the form of three $Fe_4 - S_4$ clusters (Jordan et al. 2001). Cytochrome oxidase contains three Cu atoms and two Fe atoms made from its haem a and a_3 sites (Ferguson-Miller and Babcock 1996)

correct tetrapyrrole cofactor, which is much less challenging than discerning the individual elements alone.

2.2 The Liberators of Dioxygen Altered Metal Speciation in the Environment

During early evolution, prior to the development of photosynthesis, life was restricted to habitats where energy could be derived from inorganic chemical sources. Molecular phylogenies imply that the early ancestors of cyanobacteria arose approximately 2.7 billion years ago (Brocks et al. 1999) and underwent a rapid adaptive radiation, presumably due to the greater autonomy afforded by photosynthesis and hence the opportunity to colonize previously vacant niches. This coincided with a global rise in di-oxygen as a by-product of the activity of the water splitting enzyme, changing many habitats from anaerobic to aerobic. In turn, this altered the chemical forms and solubility of metals with, for example, ferric-iron becoming largely insoluble. Only small amounts of ferric-iron bound to organic molecules would remain in aerobic solution. Thus, cyanobacteria were evolving swiftly at a time when the availabilities of essential metals were changing.

2.3 Thylakoids and Cytoplasmic Compartmentalization

Cyanobacteria contain internal membrane-bound compartments, thylakoids, which house the photosynthetic machinery. Some slow-growing strains, such as *Gleobacter*, are devoid of thylakoids and photosynthetic electron transport occurs at the plasma membrane. The thylakoid lumen, at least in the model organism *Synechocystis* PCC 6803, is known to be distinct from the periplasm since a GFP-fusion to the TAT specific targeting sequence of *E. coli* TorA gives detectable fluorescence in the periplasm but not in the thylakoid lumen (Spence et al. 2003). Cryo-EM studies have observed internal lipid bodies from which thylakoid membranes emanate (van de Meene et al. 2006), suggesting independent processes of biogenesis. Such cytosolic compartmentalization provides additional opportunities for metal-partitioning in cyanobacteria, especially in relation to copper, iron, and manganese where the predominant demand lies within the thylakoids.

3 Metal Sensors

Cells somehow detect when critical metal thresholds, either excess or deficiency, have been exceeded. In bacteria, this commonly involves metal-responsive DNA-binding transcriptional regulators.