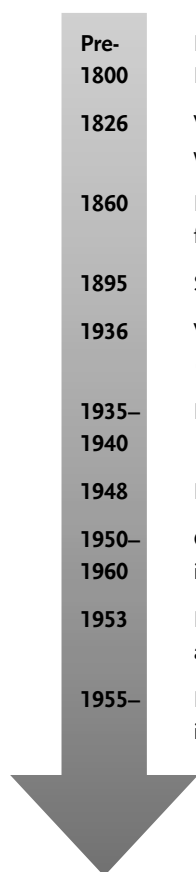


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The Biodistribution of Metal Ions

Robert J. P. Williams



Pre-1800	Lime (Ca) and phosphate (P) used in agriculture. Many elements used in medical preparations (e.g., Hg, As).
1826	Wöhler's recognition that organic chemicals H, C, N, O were in organisms.
1860	First suggestion of essential requirements for N, S, P, Ca, Mg, Fe from minerals.
1895	Spectroscopic recognition of heme in living systems.
1936	Warburg's book <i>Metal Prosthetic Groups</i> published (heme enzymes especially).
1935–1940	First isolation of zinc and copper enzymes.
1948	Irving-Williams series of transition metal stability constants.
1950–1960	General understanding of complex ion stabilities, including those of Na ⁺ , K ⁺ , Mg ²⁺ and Ca ²⁺ .
1953	First effort to understand functional value of ions in relation to above data in an article entitled "Metal ions in biological systems".
1955–	Increasing use of physico-chemical methods for study of metal ion compounds extracted from cells (now including Co, Ni, Se).

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1.1

Introduction

An obvious limitation to the biodistribution of metal elements – and one which will be removed very quickly from consideration – is that the process is clearly restricted by the availability of such metal elements in the environment (see Table 1.1). Availability itself is limited by ambient *aqueous* conditions, even if the system within any locality is at equilibrium. The limiting equilibrium conditions are governed in part by the pH and the redox potential in particular local environments, and can vary from a very low (acidic) pH to alkali values, and from a virtually zero oxygen equilibrated potential of less than -0.4 V (which is that of the hydrogen electrode at pH 7.0) to one of $+0.8$ V (which is that of the oxygen electrode potential at the same pH). An abundance of solubility product and complex formation equilibria under these conditions, and assuming a pressure of 1 atmosphere and a temperature of 0 to 30 °C, make it possible that some 30 elements could be taken up by cells at reasonably low energy cost. The availabilities of metal elements due to these factors have changed with time (see Table 1.1 and Figs. 1.1 and 1.2). Initially, conditions on Earth were slightly less reducing than those demanded by biosynthesis, so that oxygen has continuously increased (see the arrows in Fig. 1.2), with obvious consequences.

Table 1.1 Changes with time of estimated available concentrations of metal ions in the sea.

<i>Metal ion</i>	<i>Original conditions (M)</i>	<i>Aerobic conditions (M)</i>
Na ⁺	$> 10^{-1}$	$> 10^{-1}$
K ⁺	$\sim 10^{-2}$	$\sim 10^{-2}$
Mg ²⁺	$\sim 10^{-2}$	$> 10^{-2}$
Ca ²⁺	$\sim 10^{-3}$	$\sim 10^{-3}$
V	$\sim 10^{-7.5}$	$\sim 10^{-7.5}$ (VO ₄ ³⁻)
Mn ²⁺	$\sim 10^{-7}$	$\sim 10^{-9}$
Fe	$\sim 10^{-7}$ (Fe ^{II})	$\sim 10^{-17}$ (Fe ^{III})
Co ²⁺	$< 10^{-13}$	$\sim (10^{-11})$
Ni ²⁺	$< 10^{-12}$	$< 10^{-9}$
Cu	$< 10^{-20}$ (very low), Cu ^I	$< 10^{-10}$, Cu ^{II}
Zn ²⁺	$< 10^{-12}$	$< 10^{-8}$
Mo	$< 10^{-10}$ (MoS ₄ ²⁻ , Mo(OH) ₆)	$\sim 10^{-7}$ (MoO ₄ ²⁻)
W	$\sim 10^{-9}$ (WS ₄ ²⁻)	$\sim 10^{-9}$ (WO ₄ ²⁻)
H ⁺	pH ~ 7	pH 8 (sea)
H ₂ S	$\sim 10^{-2}$	10^{-2} (SO ₄ ²⁻)
HPO ₄ ²⁻	$< 10^{-5}$	$< 10^{-5}$ (HPO ₄ ²⁻)

Note: The value for the original primitive conditions are estimates based on a pH of 8.0, an H₂S concentration of $\sim 10^{-2}$ M, and a CO₂ pressure of 1 atmosphere. The concentrations in today's aerobic condition are taken from [2]. Those of the original environment are estimated as described in [1] and [4].

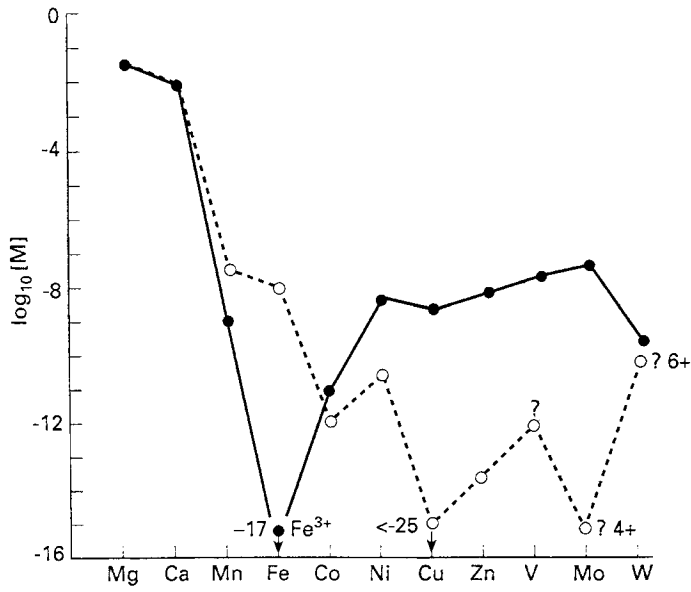


Fig. 1.1 Distribution of metal ions in the sea during the early sulfide-rich period (○) and the recent oxide-rich period (●) of the Earth's history.

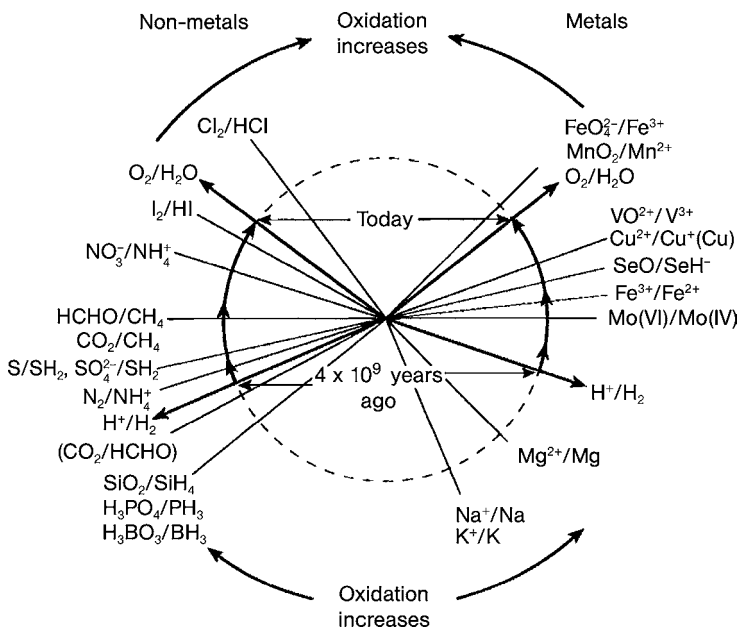


Fig. 1.2 Diagram showing the changes in oxidation state of the elements with time, from close to the H⁺/H₂ potential (initially 4 × 10⁹ years ago) to the O₂/OH⁻ potential of today.

Within cells, distribution is made more complex in that the cells must themselves control any competition between the metal ions in the same internal compartment; moreover, the metal ions must also have a functional value. Hence, some available ions are superfluous to needs (e.g., Rb^+ and Sr^{2+}) in most circumstances, as all of their functions can be undertaken by more available elements (e.g., K^+ and Ca^{2+}). Many of these competitive features can be described by equilibria within and without cells, though the cell must use energy to take in essential ions, or to reject toxic ions. Those elements that are found prominently in most cells, together with their free concentrations in the central cell compartment, are detailed in Table 1.2.

Table 1.2 Current concentrations of free metal elements in the cell cytoplasm.

<i>Element</i>	<i>Concentration</i> [<i>mol L⁻¹</i>]
Na^+	10^{-3}
K^+	10^{-1}
Mg^{2+}	10^{-3}
Ca^{2+}	10^{-7}
Mn^{2+}	10^{-7}
Fe^{2+}	10^{-7}
Co^{2+}	$< 10^{-9}$
Ni^{2+}	$< 10^{-9}$
Cu^{2+} (Cu^+)	$< 10^{-14}$
Zn^{2+}	10^{-11}
MoO_4^{2-}	10^{-8}

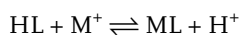
Not all metal ions in complexes are in equilibrium with the free forms in cellular solutions. It is known, for example, that porphyrin complexes do not equilibrate. Moreover, questions must also be asked about their distribution in cells, noting that the irreversible trapping of ions in porphyrins is limited to Fe, Co, Ni, and Mg, and in rare cases to Cu, together with the different irreversible complexes of Mo and W. These last two elements are quite different, since the chemistry of Mo and W is based on the uptake of anions and is therefore comparable with the irreversible chemistry of elements such as phosphorus. It must also be noted that, in general, irreversible formation refers to a complex in which the metal ion binding is broken only on destruction of the ligand. Other important features of the distribution to which we return after describing the nature of equilibrium and irreversible synthesis controls over distribution are the rates of exchange from complexes. Biological distribution has functional significance, and the use of different on/off rates has been decisive in the evolution of the selected distribution of different metal ions.

1.2 Rates of Exchange

Having distinguished between reversible and irreversible complex ion formation, it is clear that there is a vast range of exchange associated with the complex chemistry of metal ions. In fact, binding becomes effectively irreversible if the rate of exchange is much slower in one function than in another, or if all the complexes of the given metal ion do not exchange within the life-time of the compartment in which they are held. Compartment life-times vary from a few seconds to many years, so that an irreversible step must be defined by its context. Initially, this problem of rates in the description of biodistribution is ignored (the subject will be discussed later in the chapter as it is extremely important). Here, focus is centered on a discussion of equilibrium constants between metal (M) and ligand (L).

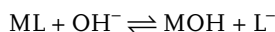
1.3 The Limitations of Water as a Solvent

Before describing the quantitative values of equilibrium constants, it is important to note two simple limitations on ligand and metal concentrations. For many anions in water, the equilibrium with H^+ controls free L and so ML:

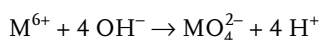


The pK_a values of RO^- , RS^- and R_2N^- are quite high, removing L as HL, but those of RCO_2^- , as well as those of simple anions such as CO_3^{2-} , SO_4^{2-} , and HPO_4^{2-} , are sufficiently low to allow binding to the metal ions – even those of the weakest acceptor character such as Mg^{2+} and Ca^{2+} .

A second equilibrium of concern is the competition for M from OH^- , especially under oxidizing conditions:



The OH^- ligand is particularly powerful in its interaction with small, highly charged metal ions, and the reactions limit external availability and L-binding in cells while the very highly charged metal ions become available for binding only as anions (e.g., MoO_4^{2-} compare SO_4^{2-}). These anions arise through extensive hydrolysis:



but note that more limited hydrolysis gives rise to oxene cations such as MO^{3+} , MO_2^{3+} , and MO_2^{2+} , which are found for several metals, including molybdenum, uranium and vanadium.

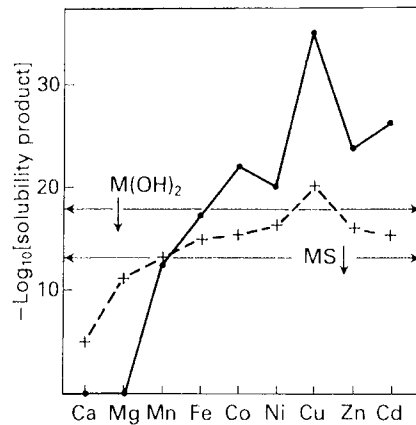
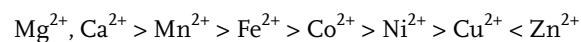


Fig. 1.3 The solubility products of sulfides and hydroxides. The horizontal broken lines refer to the division between the available (below) and the not-available (above) under the conditions of early life (H_2S , mM), and in oxygen today when only hydroxide limits availability.

The important reactions of OH^- are limited in cells to pH 7 in most compartments, but to much lower pH values in a few cases. The environmental pH of “fresh” water shows a much wider variation than the sea, some of it due to the influences of man (e.g., acid rain).

A different limitation arose early in the history of life, and persists today in some anaerobic zones, namely the solubility of sulfides in the presence of saturating solutions of H_2S . This limitation is shown graphically in Fig. 1.3 and is detailed in Table 1.1.

Thus, the solubility of salts in a given environment is a major limitation upon the possibility of distributing metal ions. In sulfide solutions the solubility decreases in the series (Fig. 1.3):

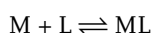


Anaerobic organisms in sulfide media have very low contents of all but Mg^{2+} , Ca^{2+} , Mn^{2+} and Fe^{2+} . In aerobic solutions, hydroxide greatly limits the availability of all M^{3+} and M^{4+} ions, including Fe^{3+} , but not greatly that of M^{2+} . Today, therefore, cells must have very considerable scavenging powers for Fe as they require it as Fe^{2+} . Fe is stored as $\text{Fe}(\text{OH})_3$, and consequently cells may well have to limit the concentration of several other divalent ions in order to avoid poisoning, especially in the cytoplasm.

Other important solubility considerations are those of Ca^{2+} , Sr^{2+} and Ba^{2+} as carbonates, phosphates, and sulfates.

1.4**Equilibrium: Values of Binding Constants**

As noted above, there is a general difference between metal ions and their complexes and non-metal elements and their compounds, in that the metal complexes inside a compartment are usually connected to exchange equilibria while the non-metal compounds are mostly under kinetic control. Here, the equilibria will be treated first. The equilibria concerned are in the simplest form, where M is a metal ion and L a ligand; these can be written as:



Here, M and L can be charged with values up to quite high numbers. In order to secure the most appropriate balance of useful metal ions, the uptake of elements of all types into cells is programmed since there is for each cell – and for each cell compartment – an optimum concentration of free and bound forms. Immediately, the question must be asked as to how concentrations of M and L can be managed to fix equilibrated ML concentrations. Such an understanding will involve the passage of metal ions in and out of the compartments, the synthesis of L, including those under genetic control, as well as the equilibrated interaction between their concentrations. The equilibria include L as a small molecule, an active protein, a buffer, a transcription factor, a transport protein, and also as a pump. Since free [M] interacts with all of these at equilibrium, it is necessary to determine how its values are arranged to meet functional needs involving all of these processes.

1.5**Quantitative Metal Ion Equilibria: Donor Strength**

The equilibria between metal ions and all types of ligands, which determine distribution in cells, have been studied for over 50 years. The values of the constants can be detailed discriminately as:

1. Donor atoms of ligands, including the size of holes in the ligand structure and electronegativity; these factors are also observed in simple lattices of salts.
2. Constrained bond lengths and bond angles at the binding site which are only seen in ligands with stereochemical restrictions.
3. Spin states, which are seen in very few simple complexes (e.g., cyanides).

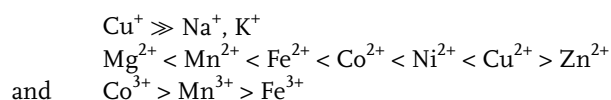
Whereas point (1) is the major term in the radial part of the ligand field, points (2) and (3) have considerable dependence on angular terms. The donor atoms of biological interest are O, N, and S and, to a much smaller degree, C and Se. Thus, organometallic chemistry is of little concern. The donor strength of O, N, and S is dependent on their charged state or partial charge as binding is partly ionic and partly covalent. In all conditions the order of donor strength in water is also

dependent upon the concentration of the free ligands. In their neutral condition (i.e., R_2O , R_3N , R_2S), the competition from water (H_2O) at 55 M is such that it prevents all but weak binding. Hence, the use of terms such as “hard” and “soft” appropriate to the gas phase become of much lower general value in water solution. Thus, it is found that only those metal ions with a high electron affinity can bind simple R_3N , whereas hardly any type binds R_2S . The most polar, R_2O , can bind to a limited degree generally. Before examining the reason for these different bindings by metal ions, it should be noted that in the state RO^- , O^{2-} , R_2N^- and RS^- , S^{2-} a very different picture emerges in that all the negative O-donor centers can bind to some degree to virtually all M^+ and M^{2+} , even in the presence of water, while the sulfur donors show very selective binding (see Fig. 1.3), from very strong to very modest. Before examining other features of the nature of ligands more closely, it is important to discuss the nature of M.

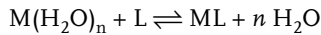
1.6

The Effect of Size and Charge of Metal Ions

Metal ions carry a variety of charges such as M^+ , M^{2+} , M^{3+} and MO^+ , MO^{2+} , but here they will be grouped into anionic forms (e.g., MO_4^- , MO_4^{2-}); the non-metal anions will be discussed later. Metal ions have a wide range of electron affinities, and those which need to be observed in relation to biological equilibria include:



Although the consequences for binding are very general, there is another major factor present, namely the size of the ions. In a somewhat obvious manner, size can be used either to allow binding or to prevent it when the ligand forms a ring such as a porphyrin or a hole in the shape of a horseshoe. Here, a given radius of one ion fits best, and selection is based largely on a combination of the size of the hole, the hydration of the cation, and the nature of the donor ligand atoms. The stress on hydration energy is necessary in that the smaller cations would always bind best to any anionic or negative end of a dipole but for this energy. It is not a case of dealing with absolute scales of donor/acceptor strength or ionic binding when analyzing biological aqueous solutions, but rather with relative binding to ligands compared with binding to water molecules. Consider first a hole size of a ligand which fits the Ca^{2+} ion (1.0 Å radius) against binding to a Mg^{2+} ion (0.60 Å radius). In absolute terms, no matter what the ligand donor atoms or their symmetry, Mg^{2+} will bind better through simple electrostatics. If water at 55 M is now introduced as a competing ligand, six water molecules (which are quite small) fit very neatly around Mg^{2+} and, although Ca^{2+} binds seven or eight water molecules, water binds much more weakly. The overall equilibrium with this ligand hole:



can be such that Ca^{2+} binds 10^4 -fold more strongly. This is seen in the arranged distribution of Ca^{2+} against Mg^{2+} , not only in the walls of cells but also especially in vesicle cell compartments of eukaryotes and in the short life-time binding of Ca^{2+} when it acts in the cytoplasm as a trigger of muscle action. Clearly, the extent of binding depends critically upon the concentration of the metal ions in a given compartment. Where they are equal or close to 10^{-3} M outside the cell, the binding constants are managed such that for Ca^{2+} it is close to 10^4 and for Mg^{2+} it is about 10^2 M^{-1} . In storage vesicles, the $[Ca^{2+}]$ is about 10^{-3} M, and there is little Mg^{2+} so that a binding constant of 10^3 M^{-1} to Ca^{2+} is adequate. Hence, in both circumstances only Ca^{2+} is bound. In the cytoplasm, Mg^{2+} is 10^{-3} M, but Ca^{2+} – before a triggering input – is less than 10^{-7} M (Fig. 1.4). A trigger protein will operate as long as its binding constant is 10^6 M^{-1} when the triggered Ca^{2+} input to the cytoplasm exceeds 10^{-6} M, and provided that the Mg^{2+} binding constant is less than 10^3 M^{-1} . This is the condition of a huge variety of calcium and magnesium binding constants inside the cell cytoplasm of all eukaryotes, for example calmodulin, S-100 proteins, carrier (chaperone) proteins, and buffer proteins. These proteins are virtually free of calcium for most of the time, and Ca^{2+} ions are seen to be distributed at such high concentration as 10^{-6} M only in bursts.

The static distribution of free Ca^{2+} ions relative to free Mg^{2+} ions is clearly managed by pumps which maintain the free $[Ca^{2+}]$ low in the cytoplasm but keep it high in extracellular fluids and in stores in vesicles. The pumps operate

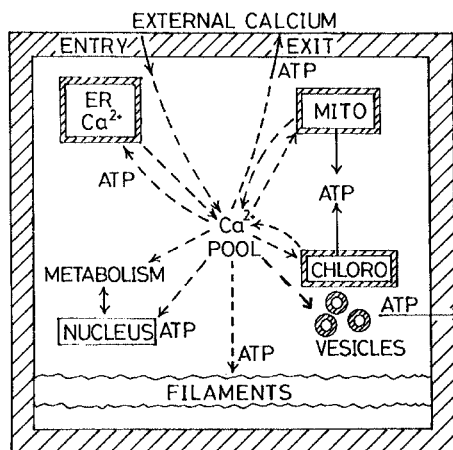


Fig. 1.4 The distribution of free and bound Ca^{2+} in cells. The external endoplasmic reticulum (ER) and vesicle free Ca^{2+} concentrations are ca. 10^{-3} M; bound Ca^{2+} concentrations may be much higher (e.g., in minerals), whilst the cytoplasmic concentration is 10^{-7} M at rest and can be triggered to 10^{-6} M. ATP is involved in energy utilization.

at two different equilibrium values, with one side internal to the cytoplasm with a 10^7 M^{-1} binding constant pumping outward and one of only 10^3 M^{-1} external to the same compartment; thus, influx is not easy. The result is a gradient of 10^4 between the outside (and in vesicles) and inside, and this has extreme functional value. Mg^{2+} has quite different pumping constants to control its concentrations. Note, however, that all binding sites in any one compartment must be similar so that all can function at the same time – that is, constants of trigger proteins, buffers, stores, inside surfaces of pumps and any transcription factors in the same cytoplasmic compartment (see below).

The value of this control over free static $[\text{M}^{2+}]$ can be seen in the case of the functions of Mg^{2+} in the cytoplasm. The concentration there of 10^{-3} M matches closely all its binding constants of slightly greater than 10^3 M^{-1} with simple anions such as pyrophosphate in various mononucleotide polyphosphates (e.g., ATP) and many proteins (Fig. 1.5). Ca^{2+} binding strength is similar or even slightly greater, but now it is not functional as $[\text{Ca}^{2+}]$ is 10^{-7} M . Mg^{2+} is often found distributed uniformly in the extracellular and cytoplasmic spaces, but is absent from many vesicles.

Pumps with similar binding constants internally and externally to the respective proteins found in the binding compounds control the distribution of all cations

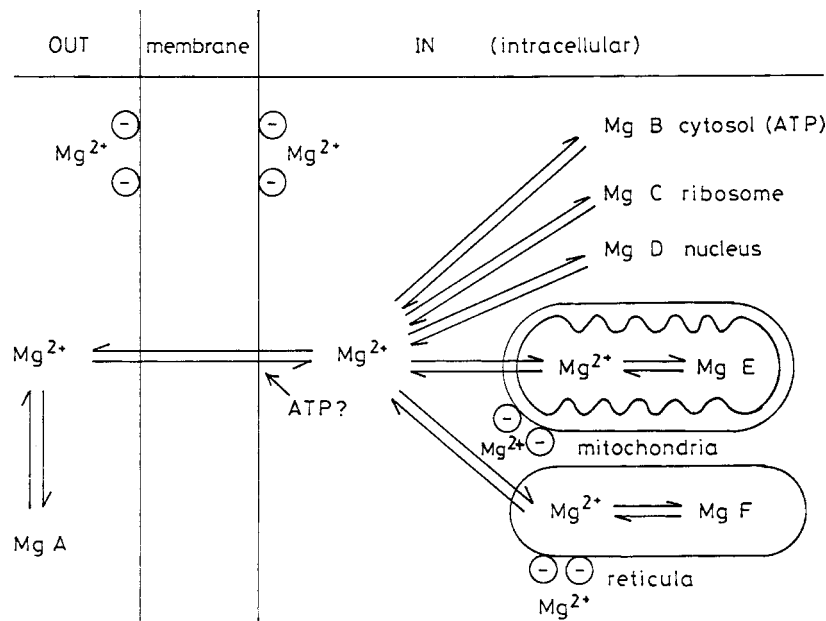


Fig. 1.5 The distribution of free and bound Mg^{2+} in cells. Environmental concentrations may often be 10^{-3} M (or even higher as free Mg^{2+}), and is 10^{-3} M in all cytoplasmic compartments (both free and bound), except for vesicles and the ER, where concentrations are much lower. Note how the equilibria assist homeostasis. In plant chloroplast membranes Mg is highly concentrated in chlorophyll.

(and anions) in fast exchange. For example, K^+ is 10^{-1} M while Na^+ is 10^{-3} M in the cytoplasm, so that K^+ binds to DNA and RNA (as does Mg^{2+}) but Na^+ does not (compare Ca^{2+}). Note how it is now the larger cation which is distributed inwardly in contrast to the use of the smaller Mg^{2+} . This distribution is not based simply on chemical usefulness but is essential to provide protection against excess Na^+ and Ca^{2+} . The contained external solutions are usually high in Na^+ but low in K^+ (e.g., in blood).

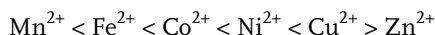
Whilst pumps control the concentrations of M in the compartments, control of the free ligand can be assessed later. The above account of Mg^{2+} cellular chemistry did not include the case of Mg^{2+} chlorophyll, in which Mg^{2+} does not equilibrate with free Mg^{2+} ions and where distribution concerns a membrane phase rather than an aqueous solution. Chlorophyll is, of course, only found in plants and certain bacteria and not in animals or fungi, this being a powerful indication that biodistribution is highly dependent on genetic expression. However, the major distributions of Na^+ , K^+ , Mg^{2+} , and Ca^{2+} in the aqueous phases of all phyla are readily described.

1.7

The Effect of Electron Affinity

Bearing in mind the situation regarding equilibria, attention is now focused on cations of the series Mn, Fe, Co, Ni, Cu, and Zn. Here, the concern is mainly with covalent contributions (see the series of sulfide equilibria in Fig. 1.3) and, to some degree, with symmetry-dependent (ligand field) energies. It should be observed immediately that the central field covalency, and not the angular dependence, is dominant and that there is always competition involving water molecule binding to free ligands. Although the size of the ions does not matter greatly here, it should be noted that Mn^{2+} (0.75 Å) is considerably larger than Ni^{2+} (0.60 Å). On occasion, the strong electrostatic term is important, and renders any simplistic theoretical discussion of soft and hard donor/acceptor binding susceptible to mistakes. (Many textbooks confuse much of the description of aqueous equilibria by placing rather naïve theory before facts.)

The major obvious observation is the equilibrium binding to most donors in the Irving–Williams series (Fig. 1.6):



This fundamental empirical series is a major feature of the geochemical distribution of these free ions (see Fig. 1.3) – that is, their availability – but it is equally important with regard to their biodistribution. The series is a dominant factor in what could be achieved in the evolution of the chemistry of organisms, for the equilibria are very restrictive conditions. An examination of binding to different organic molecules suggests that there is little concern with binding other than to multi-dentate chelates (contrast Mg^{2+}).

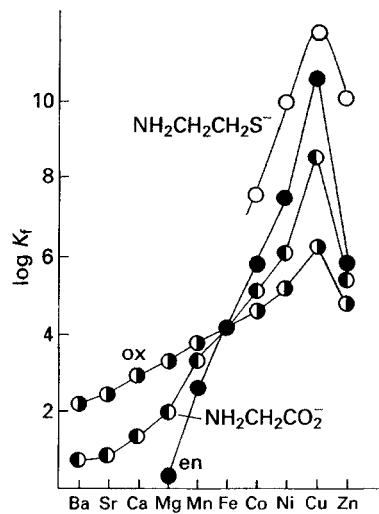


Fig. 1.6 The stability constants of some complex ions showing the Irving–Williams series. Note that given differential control of free ion concentrations is maintained by pumping. The selectivity shown here allows the distribution of all ions in specific complexes (e.g., Mg^{2+} with ATP and Cu in redox proteins).

Figure 1.6 provides the binding constants to a series of ligands with different binding atom donors, as well as some shape-selective features. These have been chosen to illustrate the magnitudes and slopes of the equilibrium constants as atomic number of the metal ion changes. These data apply to simple ligands and also to proteins in the cytoplasm of all cells. The striking features are:

- the very strong slope of the plots for thiolate donors with very poor binding to the weakest metal Lewis acids Mg^{2+} and Ca^{2+} ;
- the very equal small binding constants for all O-donors of low $\text{p}K_a$; and
- the intermediate behavior of slope and absolute value of binding constants for N-donor ligands.

Given these equilibrium controls over binding and the constraints on free M by the pumps in and out of cell cytoplasm which, for example, restrict free $[\text{Mg}^{2+}]$ to 10^{-3} M and free $[\text{Cu}^{2+}]$ ($[\text{Cu}^+]$) to 10^{-15} M (Fig. 1.7), there is no chance of Cu^{2+} (Cu^+) binding to an O-donor site which can accept Mg^{2+} , and no chance of Mg^{2+} binding to an S- or an N-donor site which can accept Cu.

This combination of equilibrium and concentration controls applies differentially to all metal ions, thereby providing the basis for selectivity. The selection is managed then in part by donor strength and in part by steric considerations. These generalizations are in accord with the observation that the separation of metalloproteins from the cell cytoplasm yields each metal ion with one type of protein for strong discriminatory binding of Fe^{2+} to Zn^{2+} and often no Mg^{2+} bound

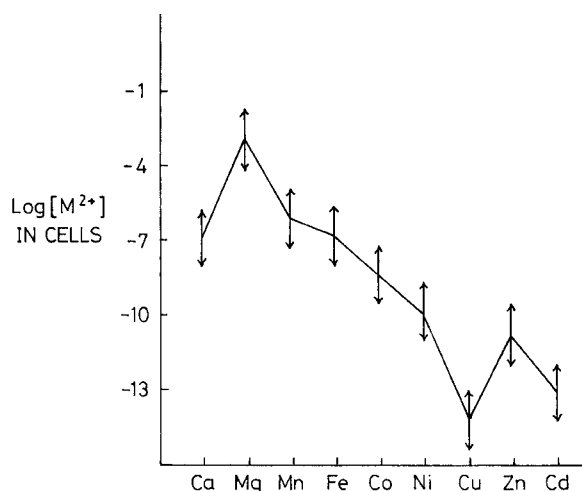


Fig. 1.7 The concentration of free metal ions common to the cytoplasm of all cells: a feature of life.

to any protein. Mg^{2+} must be added back to obtain a functioning protein. It is the type of protein made available for a given metal ion which decides the local distribution in all compartments. In order to proceed to ML concentrations, it is necessary to outline the production and distribution of the binding proteins. Before doing so, the reader will have observed that the distribution discussed is of monovalent or divalent cations. The three or four charged cations are little used in cells since they have very strong (effectively very slow) exchange properties, they can block the binding of Mg^{2+} and Ca^{2+} , and if their presence is not stopped by insolubility outside, the ions are rejected. Thus, metal elements of Groups 3, 13 and 4 and 14 of the Periodic Table are not distributed. Fe^{3+} is used in stores and in some clusters, but is released as Fe^{2+} except in external enzymes. Other metal ions that are not often used are also generally toxic, including Sn^{2+} , Pb^{2+} , Cd^{2+} , and Hg^{2+} , and these are usually pumped out of the cells. Thus, biological cells distribute many metal ions in the cytoplasm so as to include the full variety of the chemical properties of the Periodic Table, while removing the threat from any possible competing ions. Those found in many cells are seen to include elements with virtually all functional possibilities:

Na Mg •
 K Ca V (Cr) Mn Fe Co Ni Cu Zn •
 (Sr) Mo •
 (Ba) W •

In itself, this opens the possibility for man to use some 30 to 40 metal ions in drugs, but this potential has been studied to only a minimal extent.

By placing pumps in vesicle membranes the distribution of free metal ions can differ dramatically, as it is in extracellular contained fluids. The cases of Mg^{2+} and Ca^{2+} , and of Na^+ and K^+ , have been noted, but free concentrations – especially of Zn^{2+} and Mn^{2+} , as well as of Ca^{2+} – are often noticeably raised in these compartments.

1.8

Control over Ligand Concentration

Control over ligand concentrations in cells is maintained via the kinetics of gene expression of proteins, followed by transfer as the free or bound form to particular compartments. The proteins include those which bind the metal ions and those which produce small molecules for scavenging or binding these ions, both inside and outside cells. Hence, genetic control is at the heart of biodistribution and is far from simple, although it is possible to start from the simplest gene structure where a promoter is placed ahead of the proteins concerned in the DNA code (Fig. 1.8). The proteins expressed in the cytoplasm under one promoter can include an entire series from those responsible for scavenger synthesis (siderophores), pumps, carriers, and enzymes. The promoter can be under more than one control, but a common approach is via a single transcription factor which binds the metal with the same binding constant as that which is active in the cytoplasm. Hence, the binding constants of transcription factors follow the above Irving–Williams series and operate near to equilibrium. The cell is therefore controlled homeostatically in M , by feedback pumps by the ligands, L , to feedback synthesis based on metal ion-binding transcription factors, and by equilibrium constants giving an active

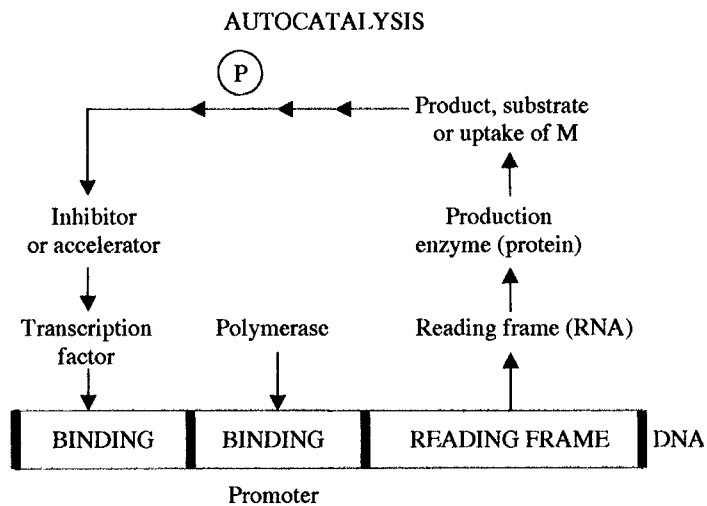


Fig. 1.8 An outline of genetic structure with feedback (F).

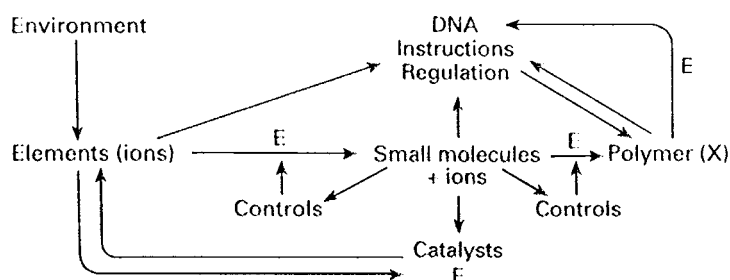


Fig. 1.9 The broad relationship between the gene, DNA, uptake of M, and cytoplasmic factors. E = enzyme or pump.

ML which may be linked to further controls (Fig. 1.9). The controlled production of ligands helps to ensure that there is no confusion in the binding of some ten metal elements (Na, K, Mg, Ca, Mn, Fe, Co, Ni, Cu, and Zn). Now while the distribution of *free* M^+ is therefore closely (but not exactly) fixed in every cell cytoplasm, the concentration of ML is not fixed, as there is a requirement for L production of an active gene system without which no specific L is produced. The value of $[ML]$ is such that there is very little excess M and very little excess of apoproteins. Small variations in transcription factor binding constants – perhaps varying ± 10 -fold – can lead to 100-fold differences in $[ML]$ for the same $[M]$. The production of small molecule ligands can also be controlled by feedback of the molecule concentration on the enzyme, itself produced under feedback control from free M (e.g., in Fe scavenging).

This is a simplified picture, as the effects of one transcription factor can be dependent on several others. Note in particular that Fe^{2+} concentration is linked not only to the Fe uptake regulator (FUR) and to the (formally) nitrate/formate regulator (FNR), but also to various kinase, phosphorylase gene and enzyme controls. In fact, Fe is then linked into a change of expression of oxidative/reductive states so that the distribution of free Fe is central to cell activity. Of equal consequence is Mg binding in the cytoplasm, though this is not so much directly linked to transcription factors but indirectly to controls through the activity of many phosphorylation steps that are absolutely essential to cell metabolism and largely Mg ion-dependent. In cells there is a vast variety of controls so as to maintain homeostasis – that is, the distribution of all active species in the cell (Table 1.3).

It should be observed also that the pumps form an essential part in biodistribution in that they are controlled by feedback from the metal ion concentration. This is in parallel to the self-induced feedback control of substrate concentration. The pumps cease import or export when the free metal concentration reaches that of the inverse of the equilibrium binding constant in the cell. The binding constant at the pump is then close to that of all other bindings (e.g., $10^7 M^{-1}$ for the Ca^{2+} exit pump). The pump requires energy and may be driven by ATP or by exchange; for example, H^+ or Na^+ gradients drive many inputs and outputs. These pumps are placed not only in the cytoplasmic membrane but also in the membrane

Table 1.3 Examples of elements used in early controls.

Element	Control (mode of use)
H	NADH (NADPH), mobile coenzymes
e/H ⁺	Thiolate \rightleftharpoons disulfide (thioredoxin)
C	CoA (acetyl is the C-fragment), mobile coenzyme
N	Glutamine
P	Very many NTP, cNMP, P, NDP, NMP
Mg ²⁺	Intimately involved with P (exchange)
H ⁺ (pH)	Intimately involved with P, S and proteins
Fe ²⁺ (Fe _n /S _n)	Free Fe ²⁺ in enzymes (exchange); redox processes
S	Used with Fe in Fe/S proteins; redox processes
Mn ²⁺	Free Mn ²⁺ in enzymes (exchange)
K ⁺ , Na ⁺ , Cl ⁻	Free ions acting on mechanical stress systems (H ₂ O levels or osmotic pressure)
Fe(heme)	Control in slow exchange

boundaries between compartments, where they act to control distribution in vesicles. Other pumps and complicated mechanisms are available for the transfer of the apoproteins or metal-bound proteins to these vesicles; consequently, the metal ion distribution is not simple and varies from organism to organism, from organ to organ, and from cell to cell, no matter the nature of the free cytoplasmic M concentrations which are all very similar. Thus, certain other features of transfer are detailed before describing the observed consequences.

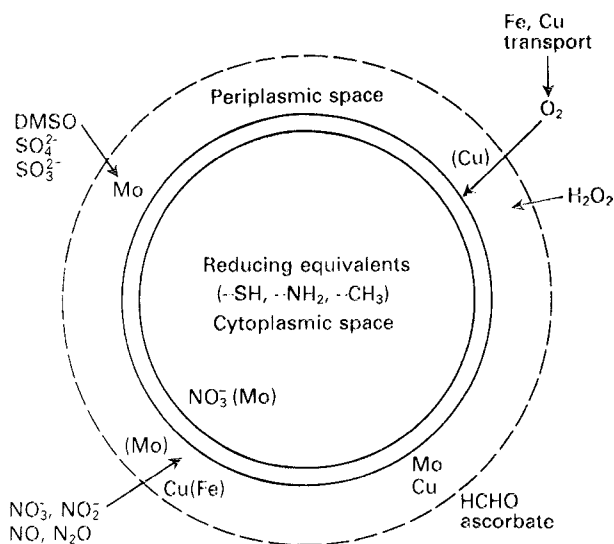
1.9

The Compartments of Organisms

In the previous sections, attention has been focused on the distribution of metal elements within the cytoplasm. The description is quite general, and complex ion equilibria in solution under the redox conditions of this cellular compartment are an inescapable feature of life. These equilibria only apply in the reducing conditions of the cytoplasm, and it has already been seen that another set of equilibria (which are changing with time; see Fig. 1.2) serve as an equal restriction on the possible uses of elements at different redox potentials (see Fig. 1.3). Although the cytoplasm protects itself from these environmental changes, or utilizes them in the way in which it binds certain elements, changing external oxidation conditions have allowed quite different element distribution and corresponding ligands in other compartments such as vesicles, extracellular fluids, and periplasmic spaces (Table 1.4). It was noted previously that bound copper became very significant in the periplasm of bacteria (Fig. 1.10) and in the vesicles in eukaryotes, whereas bound zinc was extensively utilized in the cytoplasm of eukaryotes. The proteins

Table 1.4 Types of compartment.

Compartment	Comment
Cells of different species	Plants; animals; fungi; bacteria
Differentiated cells	Cells in separate organs, liver, kidney
Cell cytoplasm	Locality of low redox potential, nucleus
Cell organelles	Mitochondria and chloroplasts
Cell vesicles	Reticula, vacuoles
Cell membranes	Only these compartments are lipid
Extracellular fluids	Blood, lymph

**Fig. 1.10** An outline of a bacterial cell with an inner cytoplasm, a membrane and an outer periplasm. Note the distribution of copper.

which are open to redox chemistry in cells, and which bind Ca^{2+} external to the cytoplasm, often have oxidized side chains. The whole basis of bioenergetics utilizes this separation of redox conditions coupled to the employment of different metal ions and ligands across membranes from the cytoplasm (Fig. 1.11). It is very important to note that, as the availability of elements changed within the environment, the biodistribution became increasingly complicated through the necessary development of increasing numbers of compartments, from bacteria to higher animals and plants (Table 1.4). At the same time, the variety of elements in minerals (Table 1.5), which are an additional compartment, was also changed. In part this was due to the ability to control concentrations of Ca^{2+} in vesicles, and of the oxidation of sulfide to sulfate.

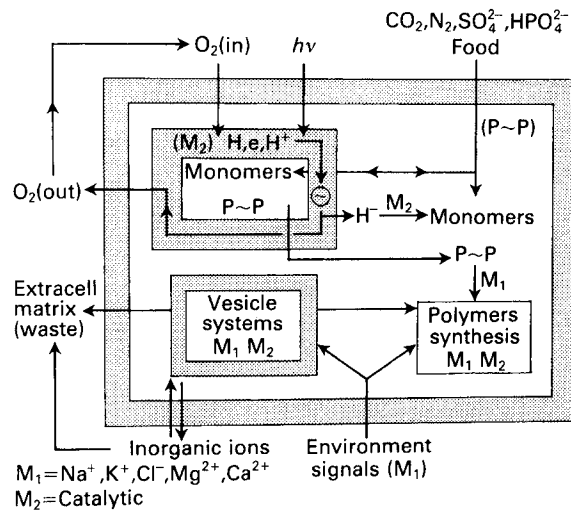


Fig. 1.11 A sketch of some features of element distribution in a eukaryote. P = phosphate.

Table 1.5 Elements in minerals.

Element	Minerals
Ca	CaCO_3 , $\text{Ca}_2(\text{OH})\text{PO}_4$, $\text{Ca}(\text{oxalate})$
Sr	SrSO_4
Ba	BaSO_4
Si	$\text{SiO}_n(\text{OH})_{4-2n}$ ($n < 2$)
Fe	$\text{Fe}(\text{OH})_3$, Fe_2O_3 , Fe_3O_4
Mn	$\text{Mn}(\text{oxalate})$ in plants
Zn	$\text{Zn}(\text{cysteinate})$ in cat's eyes

1.10

Transport

Distribution clearly involves transport. Some ions are present in high concentrations, and diffusion will quickly ensure their even distribution (e.g., Na^+ , K^+ , Mg^{2+}). However, in the case of other ions the concentration may be too low for rapid distribution, and/or the exchange from sites may be too slow for easy equilibration. The easiest case to describe is that of the movement of calcium ions through the cells of the epithelium. The calcium carrier, calbindin, transports Ca^{2+} faster than free diffusion as it is 10^{-3} M whereas free Ca^{2+} is 10^{-7} M in the cell. The selectivity for Ca^{2+} is based on generally understood principles, seven O-donor groups, and the size of the ligand-binding hole. The need for different compartments is essentially to keep the different redox conditions separated.

1.11

The Irreversible Binding of Fe, Co, Ni, Mg, and Mo (W)

When considering the binding of any porphyrin ligand to metal ions of the Irving–Williams series, it is observed that without controls the affinity for Cu^{2+} or Ni^{2+} would overwhelm that of Fe^{2+} , Co^{2+} , and especially Mg^{2+} . The series fails since equilibrium is not permitted here. The metal ions are inserted using metal ion carriers which are selective at equilibrium. These carriers are sometimes called “chaperones”, but these carriers and those for Ca^{2+} have long been known as chelatases. They are selective following the chemical binding preferences seen above. Each metal ion is then held in a different ML. The ML can be recognized by a second selective protein carrying an empty porphyrin based on synthesis from one basic starting structure (Fig. 1.12). The insertion of bound M from ML into the selected porphyrin may require energy, but once M (porphyrin) is formed it is then transferred further to its appropriate binding center in a protein which is selective for that porphyrin. Thus, it is found that Fe, Co, Ni, and Mg are differently bound and distributed, and effectively form four new “metal ions” as they do not exchange with other sites of their parent element. Compare sulfur in SO_4^{2-} and RS^- , where S behaves entirely differently in the two compounds. Thus, while Mg^{2+} distributed amongst binding sites in the aqueous cytoplasm is important in many ways, Mg^{2+} (chlorin) is membrane-located and is essential for light absorption. Fe in cytochromes is to some degree similarly different from Fe^{2+} bound directly to proteins.

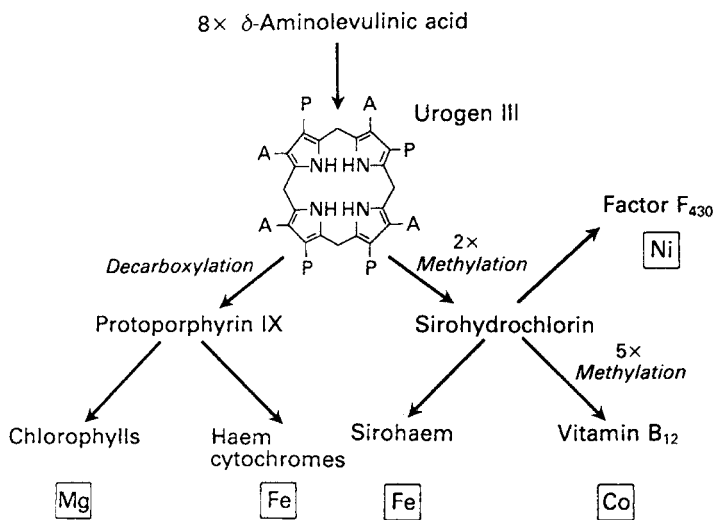


Fig. 1.12 The irreversible incorporation of elements into porphyrins; this effectively makes them quite separate entities from their free metal ions and the complexes in equilibrium with these ions.

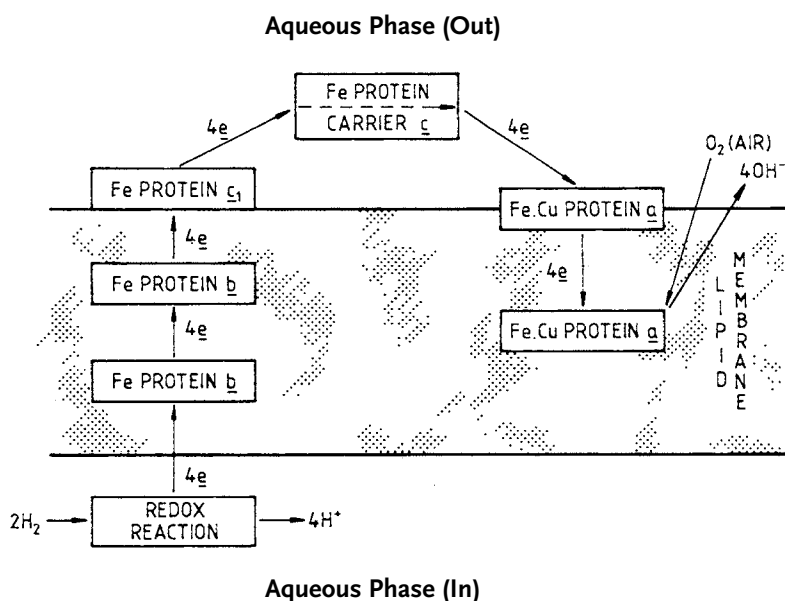


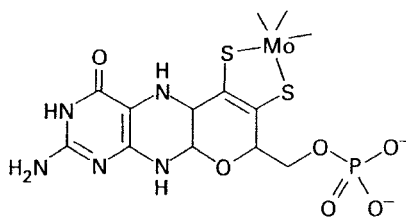
Fig. 1.13 The distribution of iron porphyrins in membranes essential for oxidative energy.

The distribution of porphyrins depends on their production, which is a genetic feature. Typically, some “red” cells are observed with abundant heme, while “white” cells have very little heme (e.g., liver, red and white blood cells, and red and white muscles). The control rests in that gene expression is again under the feedback control of transcription factors. The synthesis of heme (and of Fe_nS_n centers) takes place in the mitochondria, so that these cofactors also must be transported. As some of them are hydrophobic they now can be placed in membranes and become part of the basic units of energy distribution (Fig. 1.13). This raises again the problem of a need for carriers in order to distribute ions or porphyrins to their sites of action. A similar problem arises with the molybdenum cofactor Moco and FeMoco (although as molybdenum is handled initially as an anion, any discussion of it has been omitted at this stage).

1.12

Vanadium, Molybdenum, and Tungsten

These three metal elements occur as anions and can be handled by quite different methods from those used in the distribution of cations. Note first that tungsten is used apparently in place of molybdenum in the deep-sea sulfide-rich trenches and in some anaerobes. Their requirement is essential for O-, S- and N-atom metabolism. The requirement for vanadium is in halogenation and in some curious function in the animal species, tunicates.



M.Molybdopterin cofactor – Moco

Fig. 1.14 The irreversible molybdenum site in Moco, the coenzyme which controls much of molybdenum distribution.

The sites for oxyanion binding depend initially on hole-size and hydrogen bonding, but final binding is by condensation, as in phosphate chemistry. Here, however, the condensation is of the thioanions to provide in effect a covalent non-exchanging centre. The distribution is, where necessary, of the exchangeable coenzyme (e.g., Moco) (Fig. 1.14).

The case of the distribution of units such as Fe_nS_n and FeMoco is not fully understood, but there appears to be an insertion mechanism so that these units are very strongly bound in β -sheet proteins to thiolates.

1.13

Rates of Exchange

The biological distribution of elements is linked to function, and apart from the manner in which elements are bound (at equilibrium) in order to control osmosis (Na^+ , K^+ , Cl^-), to stabilize structures (Ca^{2+} , Mg^{2+} , Zn^{2+}), and to assist catalysis (some ten metal elements), they are used in controls. As explained above, control implies exchange where the metal ion acts as a useful carrier of information. However, a messenger can only act if its exchange is relatively fast compared with the time needs of the response. In some cases these requirements are concerned with response to synthesis when the time scale will depend on the life-time of the organism. The fastest syntheses of cells are those of the bacteria, with a life-time and reproduction rate of 30 minutes. Changes in synthesis must be relatively rapid, and it is observed that Fe^{2+} and Mg^{2+} with exchange times of around 10^2 per second are easily quick enough. (The exchange rate is given by the off rate; thus, for Mg^{2+} which has a slow on-rate of binding of 10^{-6} s and a binding of 10^3 M^{-1} the off-rate or exchange rate is 10^{-3} s. For iron, the on-rate is faster at 10^8 s^{-1} and binding is 10^6 M^{-1} , so that the exchange rate is 10^2 s^{-1} .) Ions with very high binding constants (e.g., $> 10^{10} \text{ M}^{-1}$) are not of functional value in properties needing very fast exchange. It is observed that Zn^{2+} is not used in synthesis controls in prokaryotes, but is increasingly used in eukaryotes, the more so the longer the life-time. Zinc in later eukaryotes is linked to the control of processes

such as metamorphosis with hormones such as sterols. These hormones also have binding constants of $> 10^{10} \text{ M}^{-1}$ and correspondingly slow exchange rates. From these exchange data it can be seen that, while Mg^{2+} and Fe^{2+} are distributed universally among all organisms and utilized in exchange controls, Zn^{2+} is only present in high, bound concentrations in eukaryote controls of synthesis. Some 5–10% of the expression of advanced plants and animals is dependent upon Zn proteins.

A quite different consideration of exchange relates to the need to obtain knowledge regarding the external situation of an organism or a cell of a multicellular organism. Here, distinction must be made between organisms which protect themselves by the use of a discrete wall that in turn limits their ability to respond (e.g., bacteria), and those organisms able to respond to external opportunity by changing shape or by rapid movement. The latter organisms include all eukaryotes which, as explained above, use the exchange of Ca^{2+} at an outside/inside exchange rate at its fastest at 10^{-3} s (on-rate 10^{-9} s , binding constant 10^6 M^{-1} , exchange rate 10^{-3} s). Hence, the high distribution of calcium in vesicles is also used as an amplifier. A multicellular organism requires faster cell–cell transmission in order that single cell changes, limited in change to 10^{-3} s by the Ca^{2+} exchange rate, can be coordinated. For this purpose, higher animals use Na^+ , K^+ and Cl^- exchange in nerves. These ions bind very poorly and have exchange rates close to 10^{-9} s , so that the response times are limited by diffusion. It can be seen that Na^+ , K^+ and Cl^- are distributed in the extracellular and cellular fluids to utilize this possibility. The rates of nerve, Na^+/K^+ , processes are much faster than the mechanical changes of organisms, which cannot be faster than the exchange rate of Ca^{2+} permits, of 10^{-3} s .

The overall conclusion is that element distribution is related to functional value in particular classes of organism, where functional value is not dependent on binding strength and catalytic power but rather on exchange rate.

1.14

Summary

There is no way in which the distribution of metal ions in all phyla can be described in one short chapter (Fig. 1.15), and consequently an attempt has been made here to outline the principles of this distribution which are largely based on:

- Availability from the environment, which has changed with time, and is believed to have driven evolution (see Table 1.1).
- Equilibrium binding in the environment and in separate compartments, giving major restrictions on what was possible.
- The operation of energy-driven pumps to move ions across membranes, so that some were maintained inside the cytoplasm (e.g., K^+ , Mg^{2+} , Fe^{2+} and Zn^{2+}) in free or bound forms, while others were strongly rejected to vesicles, to extracellular fluids, or to the environment (e.g., Na^+ , Ca^{2+} and Cu).

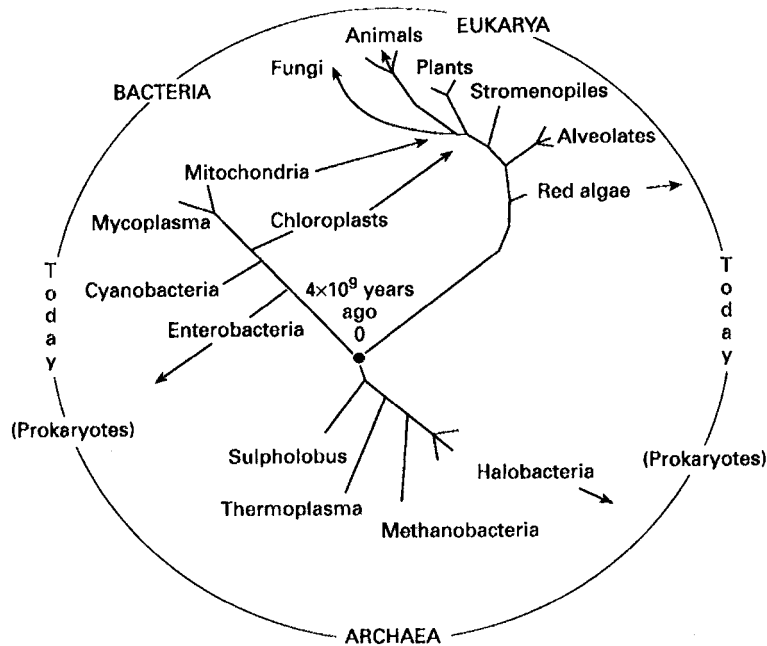


Fig. 1.15 The picture of evolution. Each organism is separate, and each has many compartments; this makes a description of the distribution of elements and their compounds difficult.

- Non-equilibrium binding to special ligands (in particular porphyrins) which allowed certain elements to be distributed in compounds in non-aqueous phases and membranes (e.g., Fe and Mg).
- All of this distribution required not simply feedback-controlled pumps (see [3]) but also feedback-controlled synthesis, such that the amounts of selected protein (and other ligands) matched the bound metal ions, leaving little or no apoproteins.

Given these principles, it can be seen how cells came to use elements differentially and functionally, with little or no confusion. These uses are seen more clearly in the other chapters of this book.

References

It is impossible to recognize the input of a vast number of people who have contributed to the studies described here. Therefore, references are provided to our own book on the subject of biological inorganic chemistry, as we believe that it covers this topic in much more detail. Additional, related books, are also listed insofar as they are connected to this chapter.

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- 2 P. A. Cox, *The Elements on Earth*. Oxford University Press, Oxford, **1995**.
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