

The Trinity of Life: The Genome, the Proteome, and the Mineral Chemical Elements

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The investigation of the nature of living organisms has been dominated recently by the study of the genome. The main thrust has been to obtain and interpret linear sequences of DNA (or RNA) nucleotides, but it is clear that an understanding of *activity* in cells can only come from the further, now quantitative, analysis of gene products, RNAs, and proteins, but especially from the detailed description of the second of these, the proteome, and the functions of its components. There is, therefore, the need to recognize that as well as the sequence we must know the structure, the local concentration in each compartment, and the activity of each protein. The activity of a biological polymer, RNA, or a protein, or even of DNA, in vivo cannot be deduced even then, since activity is a property of the whole cellular *system* and is therefore critically dependent on the concentrations of small molecules, the metallome, and ions as well as of these large organic molecules in each compartment.

Many of the small molecule and ion variables are related to the element contents of the environment so that the environment is a part of the living system. The cellular content of elements is readily divided into a dependence on the concentrations of metal ions and their complexes and on simple nonmetal compounds. Three advantages stem from the study of the metal elements relative to that of nonmetal, organic, element distributions that is of C, H, N, O, S, or P: (a) there are fewer than 20 free chemical elements to discuss (Table 1), which are easily followed in cells; (b) quantitative analysis of metal ions, free and bound, is relatively simple experimentally, even in compartments; and (c) there is a direct connection to the environment since the elements, for example free ions, are usually not grossly changed in compounds. The connection to the environment leads from the abundance and environmental availability of the elements and therefore gives an independent variable to the possibilities of evolution.

An inquiry not just into element requirements in organisms today but to an inspection of evolution linked to Earth's chemistry and its changes over 4×10^9 years is then a necessary part of the development of organisms. The nonmetals bound in small molecular species in cells, also circumscribed by the state of the elements in the atmosphere and aqueous solutions, are less easily related to their chemical elements in the environment and their changes owing to the complexity of the organic chemistry involved. Again these molecular species are directly related to the need to produce genetic material and proteins. Obviously the genome and the proteome and their evolution can also be followed but they are not directly related to environmental chemicals though they are interactive with them.

There is then a trinity of linked variables in the evolution of organisms, the genome, the proteome, and the environmental elements. Given their advantages we shall concentrate on the metal elements and their changes both in the environment and in cells. Thus we need a name for the profiles of the metal contents of cells. In each compartment we shall refer to the *free metallome*, the profile of free metal ion concentrations, $[M^{n+}]$, and the *total metallome*, which includes concentrations of free and bound metal species (1). To see the connection between the origin and evolution of the metallomes of cells to the environment and its changes we give first a brief outline of the known or presumed initial environment and its changes over time.

Table 1. Available Element Concentrations in the Sea As They Changed with Time

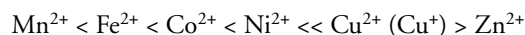
Element As Simple Ion or Species	Original Conditions/M	Aerobic Conditions/M
Na ⁺	$>10^{-1}$	$>10^{-1}$
K ⁺	$\sim 10^{-2}$	$\sim 10^{-2}$
Mg ²⁺	$\sim 10^{-2}$	$>10^{-2}$
Ca ²⁺	$\sim 10^{-3}$	$\sim 10^{-3}$
V	$\sim 10^{-7.5}$	$\sim 10^{-7.5}$ (VO ₄ ³⁻)
Mn ²⁺	$\sim 10^{-7}$	$\sim 10^{-9}$
Fe	$\sim 10^{-7}$ (Fe ²⁺)	$\sim 10^{-17}$ (Fe ³⁺)
Co ²⁺	$<10^{-13}$	$\sim (10^{-11})$
Ni ²⁺	$<10^{-12}$	$<10^{-9}$
Cu	$<10^{-20}$, Cu ⁺	$<10^{-10}$, Cu ²⁺
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)
S	$\sim 10^{-3}$ (H ₂ S)	10^{-2} (SO ₄ ²⁻)
HPO ₄ ²⁻	$<10^{-5}$	$<10^{-5}$

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 one atmosphere. The concentrations in today's aerobic condition are taken from Cox (2). Those of the original environment are estimated as described in references (1, 3, 4).

The Environment during Evolution

Prior to the beginning of life, say 4×10^9 years ago, the atmosphere was reducing as a result of the dominant universal *abundance* of hydrogen over oxygen. The most common nonmetal elements were therefore present mostly as hydrides, for example, oxygen (H_2O), sulfur (H_2S), and selenium (H_2Se), some carbon (CH_4) though most of it was CO or CO_2 , and perhaps some nitrogen (NH_3). Of the other abundant nonmetals, silicon and phosphorus were present as silicate and phosphate in rocks and in solution while chlorine was reduced to chloride (HCl), which dissolved easily in water. The HCl was neutralized by reaction with basic metal minerals and much Na , and some K , Mg , and Ca were liberated into the sea. The amount of HCl seems to have been limiting in these reactions, hence it was the abundance of Cl relative to that of other elements that decided the nature of the sea to this day. As a result of these neutralization reactions the pH of the sea settled to a value close to 8 through buffering by carbonate, phosphate, and the mixed oxides present. The reduced atmospheric and neutral aqueous solution chemistry of the nonmetals did not greatly influence the state of the most common metals, sodium, potassium, magnesium, and calcium, which give simple ions in solution,

though much of Mg and Ca also formed somewhat insoluble precipitates, such as carbonates. Formation of these carbonates presumably limited the content of these two ions in the sea. The initial presence of H_2S (probably around 10^{-3} M) caused a drastic limitation on the availability of other elements, especially of trace metals and increasingly in the transition metal series, owing to the formation of sparingly soluble sulfides, so that the concentrations of divalent elements fell dramatically from that of Mn^{2+} (Table 1). The availability of free ions, calculated from the pH and their solubility products, fall close to the Irving–Williams order (1)



The quantity of Zn in the sea must have been very low and that of Cu negligible while there would have been around 10^{-7} M Mn^{2+} and 10^{-7} M iron as Fe^{2+} (Table 1). Note, however, that it is difficult to know the availability of iron sulfide clusters that probably formed in the prevalent conditions and are somewhat water soluble. There would also have been very little molybdenum (largely precipitated as MoS_2) but more tungsten, which is not so easily reduced from the W(VI) state to the insoluble WS_2 , and almost certainly more vanadium than molybdenum since it would have been present in more soluble species (5).

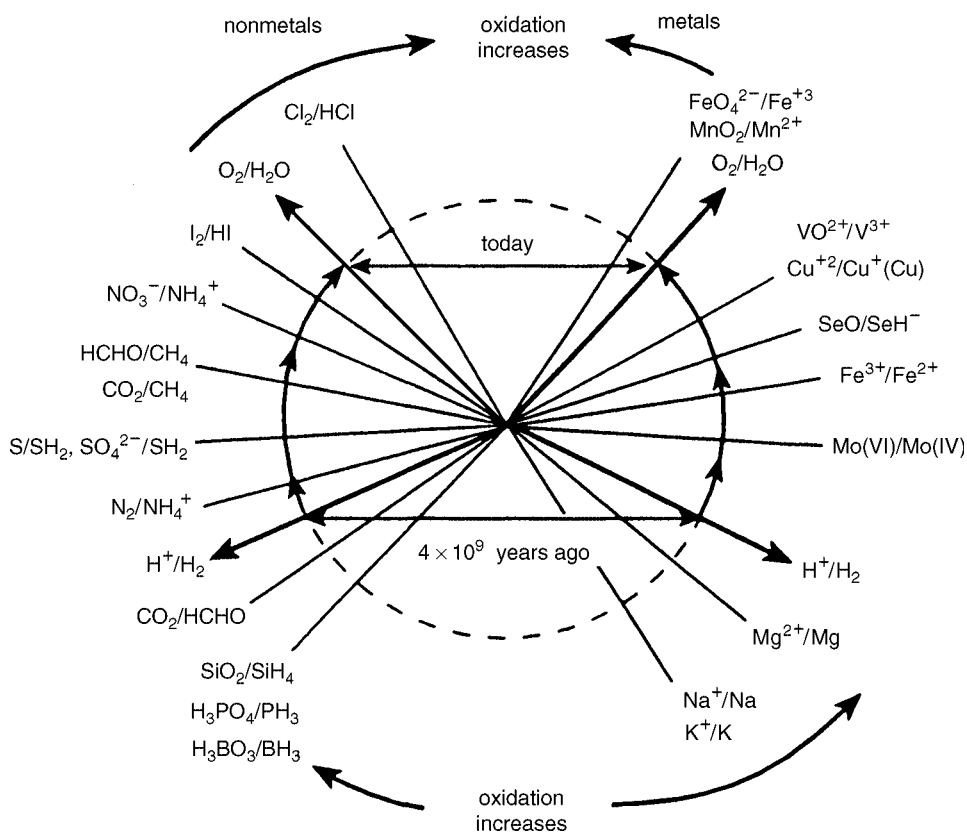
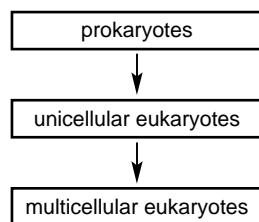


Figure 1. A diagnostic illustration of the succession of oxidation–reduction potentials showing the changes of oxidation state of different elements from an H_2 to an O_2 atmosphere. We consider that Earth's surface environment changed consecutively in the direction of the arrows over the period of 4×10^9 years. Nonmetals are on the left, metals on the right (1).

The dramatic but slow changes following the oxidation of the elements over the following 3×10^9 years as a result of the increase in O_2 in the atmosphere are shown in Table 1 and Figure 1 and have been detailed elsewhere (1, 2). Here we note first that the major nonmetals became CO_2 (C), H_2O (H), N_2 (N), SO_4^{2-} (S), and SeO_4^{2-} (Se), but there was little change in Cl^- , $Si(OH)_4$, or HPO_4^{2-} . As sulfide was oxidized to sulfate in the sea, the availability of cobalt and nickel, but much more strikingly and more recently that of zinc and of copper, inevitably and slowly increased, as did that of molybdenum (MoO_4^{2-}) and vanadium (VO_4^{3-}). However, iron availability declined rapidly as Fe^{2+} was oxidized to Fe^{3+} and $Fe(OH)_3$ was precipitated. While the slow changes were limited by the release of O_2 from living organisms, in order to gain access to hydrogen from H_2O , the consequential environmental inorganic changes are indirect and due to equilibration. The order of change in element availability therefore followed these potentials (Figure 1). The changes in the environment in turn affected the organisms in a series of ways dependent upon the timing of the oxidation of the elements. We note immediately that the major evolution of biological diversity and complexity, especially in the sequence,



closely relates to the times of these geological changes (Figure 2). For those unfamiliar with organisms, bacteria are prokaryotes (Figure 3), yeasts are unicellular eukaryotes, and modern plants and animals are multicellular eukaryotic organisms (see Figure 7). (Man is a special animal because of the way in which he uses his brain.) We shall show that these biological developments depended on the changing availability and use of the elements. Note that all eukaryotes need oxidation to produce cholesterol for their membranes; prokaryotes do not make this chemical, while multicellular organisms require extracellular oxidation products for the synthesis of their connective tissue, often associated with copper enzymes. While the first change occurred at low oxygen concentration, the second required much greater oxygen concentration.

The cellular uptake and use of elements requires energy (6). The most primitive cells may only have been able to use poor sources of chemical energy, note for example the inorganic Fe–S systems discussed by Wachtershäuser (7), but there was always the possibility of using sunlight. As this usage evolved and gave rise to O_2 in the atmosphere (Figure 2), we see also a progression in the use of other energy sources, arising from oxidation of environmental chemicals, such as sulfate and nitrate, as well as O_2 (Table 2). Against this background of changes in element availability and energy sources we can examine the development of cells from the most primitive we suppose to have existed, judging their character from that of strictly anaerobic organisms alive today (mostly archaea or related to bacteria), to higher animals and

plants. It is generally agreed that cellular life started from such anaerobic prokaryotes, cells with one central compartment and an outer membrane (Figure 3), to greater and greater compartmental complexity (see Figure 7).

The Primitive Cell Cytoplasm

The fact that the basic and major synthetic organic chemistry of all cells, the production of nucleotides, proteins, fats, and polysaccharides is reductive and is a unique feature of all organisms means that there had to be maintained reductive organic reaction pathways in the cytoplasm not only initially but to this day. These pathways are known to incorporate both phosphate and reduced sulfur into certain polymers. They are shown in all biochemical textbooks (8, 9). The inorganic content of the cytoplasm, in which these fixed pathways are found, had to be fixed since the pathways required organic reactions that needed (and still need) controlled ionic strengths, structural elements, and inorganic acid–base and redox catalysts. These needs are made up from the concentrations of metal elements that we describe by the term the cytoplasmic metallome, free and combined. Let us examine the total requirement of genome, proteome, and inorganic metal element content, especially the metallome, treating the three as an essential trinity of the flow system for all organisms. (Note we deliberately neglected the other part of the element requirement, that of the nonmetals associated with the metabolome, for the reasons given earlier.)

A first need of the primitive cell, which accumulated mainly organic *anionic molecules* (note that most biological

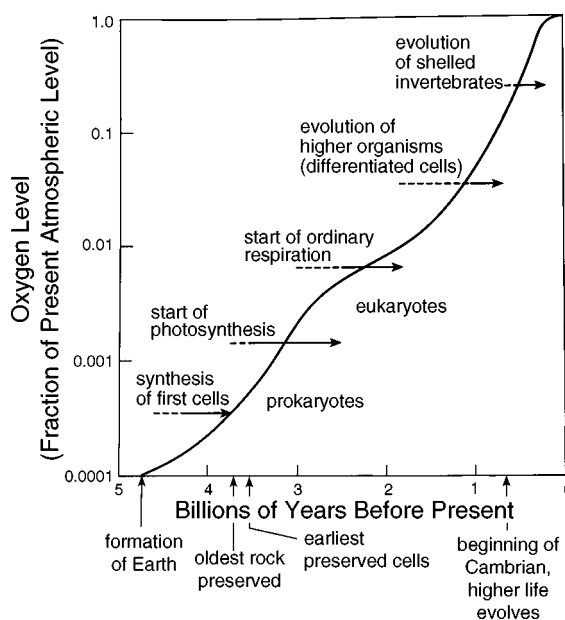


Figure 2. Evolution of the biosphere and the atmosphere: a summary of the biological and geological evidence that suggests how oxygen levels in the atmosphere may have progressed towards their present-day values and how cell organization evolved concomitantly. The availability of chemical elements changed as in Figure 1. Present atmosphere level of oxygen is 21%. (Based on Cloud, P. *Scientific American* 1983, 249 (3), 132)

molecules, for example, DNA, RNA, ATP, lipids, many proteins, and small molecules of the metabolome are negatively charged at pH 7) must have been both a controlled total osmotic concentration and an electric charge balance. If the high sodium chloride content of the sea, the major osmotic salt, had been allowed into a cell, the excess osmotic pressure would have caused the cell to burst. It is observed that there is an enforced, energy-requiring, rejection of Na^+ and Cl^- from the cytoplasm of all cells as a means of lowering the total osmotic pressure to a value close to that of the sea. In order to balance the negative electric charge, which would have caused the cell to break as a result of electrostatic repulsion, the potassium ion was allowed into the cell at concentrations higher than that in the sea. Again Ca^{2+} had to be rejected since the levels in the sea, $> 10^{-3}$ M, would have caused precipitation of many of the organic anions required in the cell. The same problem did not arise with smaller divalent cations, such as Mg^{2+} and Fe^{2+} , which do not form such insoluble salts with organic anions. Probably the somewhat larger Mn^{2+} cation was also largely rejected from cells. The necessary separation of some common elements, Na^+ , Ca^{2+} , Cl^- , away from the cytoplasm and the acceptance of others, K^+ , Mg^{2+} , and HPO_4^{2-} , to maintain a fixed ionic medium for the organic reaction system is observed to this day in all organisms all the way up to mankind. The requirements are fully described in textbooks of physiology (10). These physical-chemical demands are independent of a code (DNA or RNA) or of its particular products, much though they needed to be connected to them in a reproductive form of life. The metal element requirements are obviously related to element availability and equilibria in the environment (1).

The need to carry out difficult organic reactions at ambient temperature in water meant that cells also required powerful catalysts. Now, organic chemicals were and are not sufficiently reactive to activate the environmental small molecules for organic syntheses, such as CO_2 , CO , H_2 , and N_2 , or to manage many further synthetic steps of their reaction pathways, either oxido-reductive or hydrolytic (Table 3). Moreover only certain metal ions were both available and not poisonous to the reactions. They include Mg^{2+} and the somewhat less available transition metal ions, originally mainly iron (and perhaps some vanadium, tungsten, and manganese; see Table 1), which could have been the original set of metal catalysts. To bring about the required catalyses the very different availability and functional value of the two major ions, Mg^{2+} and Fe^{2+} , meant that they could be employed differently. Anionic centers based on O-donors such as phosphate or carboxylate that often need to be activated in condensation and hydrolytic reactions, bind M^{2+} metals weakly and only Mg^{2+} , not Fe^{2+} , was present in sufficient quantity to bind to them under the cytoplasmic conditions (Table 1 and Figure 4). Fe^{2+} could bind strongly to N- or RS-donors in organic molecules or on surfaces that could be used in redox metabolism or in energy capture, but Mg^{2+} could not. It is not surprising, therefore, to find that in any primitive prokaryote chemical system of reactions, weak solutions of Fe^{2+} , bound selectively, dominated catalysis of reductive redox steps in reactions and in energy capture based on strong binding to organic amines, sulfides, and thiols (1). Meanwhile magnesium at much higher concentration, and also bound selectively, dominated the less demanding catalysis of acid-base reactions of con-

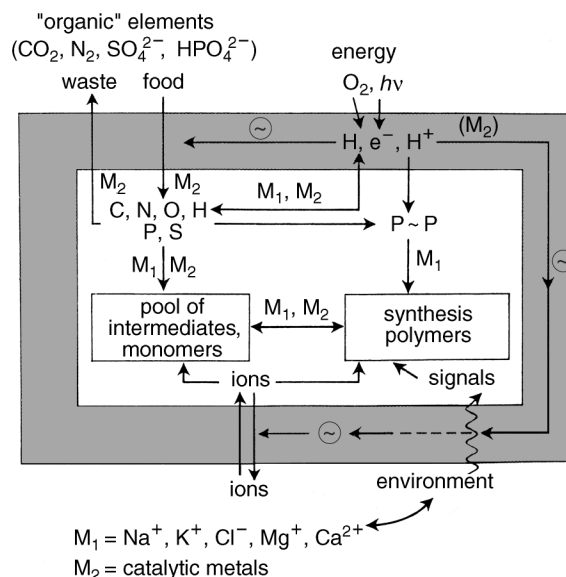


Figure 3. An outline of a prokaryote cell with a single outer membrane. Note the roles of nonmetals and metals and of energy. P~P is energized phosphate as in ATP. Note that light and oxygen as sources of energy arose in sequence.

Table 2. Sources of Energy

Period (years ago)	Energy Sources
Initial (4.5 × 10 ⁹)	(a) the sun (b) basic unstable chemicals in the crust (c) chemicals stored at high temperature in the core (geothermal energy)
After 1 billion years (3.5 × 10 ⁹)	(a) as above (b) some oxidized materials, some SO ₄ ²⁻ ; very little O ₂ , H ₂ O ₂
After 2 billion years (2.5 × 10 ⁹)	(a) as above (b) further oxidized materials; modest O ₂ , H ₂ O ₂
After 3 billion years (1.5 × 10 ⁹)	(a) as above (b) further oxidized materials, almost 1% of final O ₂ pressure
Today	(a) as above; see Figure 2 (b) man's fuels (c) atomic energy

NOTE: See Chapter 5 of ref 1 and ref 6.

Table 3. Early Requirements for Inorganic Elements

Element	Reaction Required
Fe	Electron transfer (Fe-S proteins)
Fe (Ni)	H ₂ activation, hydrogenases
Mg	Phosphate reactions, kinases, ATP activation
V (Mo)	Nitrogen activation (nitrogenase)
W (Mo)	Oxygen-atom transfer
Co	Sugar transformations, ribonucleotide reduction
Zn	Condensation (RNA polymerases)

NOTE: See Chapters 4 and 7 of ref 1.

denation and hydrolysis, based on weaker O-donor coordination. We observe that magnesium is linked especially to phosphate in energy transfer (ATP, ADP) and metabolism, and in the stabilization of RNA and DNA (1). Assuming this chemical system of catalyses is trapped in the interior of the primitive cell, its cytoplasmic free element composition, the *free metallome*, probably had a not too distant relationship to the content of its environment governed respectively by a reductive atmosphere and the ocean (Table 1 and Figure 4). We stress that zinc, nickel, cobalt, and molybdenum were only of minor use to these very primitive cells owing to their low availability from their sulfides. The dominant metal elements as free ions, that is other than in organic compounds, were therefore Na^+ , K^+ , (Cl^-) , Ca^{2+} , Mg^{2+} , and Fe^{2+} , which remain at the same free ion concentrations as cytoplasmic constituents of virtually all cells to this day. Undoubtedly other elements such as Mn, W, and V also played minor particular roles. It is in large part these fixed concentrations in all cellular systems that allow us to assert that it is a *trinity of components* that define life's chemistry, that is, these elements in primitive cells, the genome, and the proteome. The *whole system* of organisms must clearly include the chemical environment as well as a source of energy as limiting factors.

To appreciate in detail these (and later) separations among the elements, Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Fe^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} , we must look at a larger set of "effective" binding constants to the possible available donor atoms in organic molecules, especially of proteins in the cytoplasm and of potential anions of the geosphere both in reducing solutions. The possible ligands present allowed not only O-donors (mainly $-\text{CO}_2^-$ and $-\text{PO}_4^{2-}$) but also N-donors ($-\text{NH}_2$ and imidazole) and S-donors (S^{2-} and RS^-) to make coordination complexes or precipitates. In Figure 5 we plot their absolute stability constants. (The effective, or conditional binding constants are the values of binding calculated

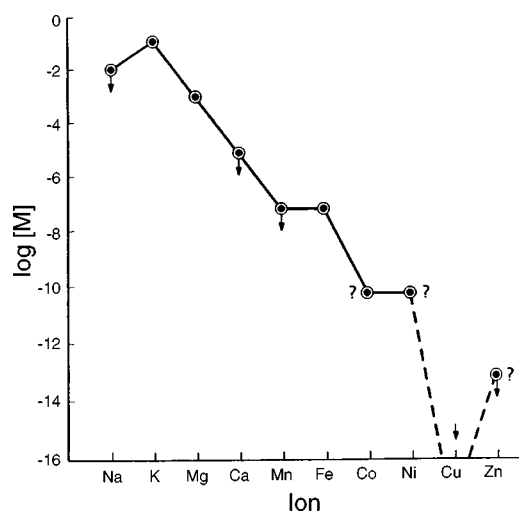


Figure 4. A plot of the logarithm of the free metal ion concentration in the cytoplasm probably for all cells. The downward arrows indicate that the value may have been somewhat overestimated. There is considerable uncertainty about the smallest values (1).

from these values under the conditions of pH and ionic strength in the cytoplasm. Thus H^+ competes with the metal ions for the ligands and reduces binding by hydroxide at $\text{pH} = 7$, for example, by up to 7 log K units). We can use these equilibrium data for the quantitative description of the bindings of many of the ions in cells since the ions are invariably in relatively fast exchange.

The descriptions, given in Table 1 and Figure 4, of the free ion concentrations in the environment and in the cytoplasm are related, therefore, to our knowledge of equilibria both in the aqueous sea (sulfide, S; carbonate, O; and hydroxide, OH solubility products) and on complex ion binding constants to a somewhat parallel set of organic binding centers, S and O donors, supplemented in cells by intermediate binding by N-donors, in the cytoplasm. It is the greater discriminating ability of sulfide in precipitation as opposed to that of thiolate or amine in coordination complex chemistry with organic molecules, that largely removed ions such as Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , and Mo^{4+} from the early sea and limited the possibility of their uptake by the most primitive cells. As stated, the higher concentration of Mg^{2+} and Fe^{2+} allowed these ions to dominate the initial anaerobic cytoplasmic chemistry of cells. Meanwhile, the anionic O-donor centers of the external surfaces of cells were bound by the high concentrations of Mg^{2+} , Ca^{2+} , and probably some Mn^{2+} in the sea (apparently Mn^{2+} was largely rejected by cells). Finally, free tungsten and vanadium, more prevalent than molybdenum in the sea in these primordial times when Mo was removed as MoS_2 , also became valuable trace catalysts in cells, (Table 3; refs 5, 11, 12).

[Note that it is the physical-chemical forces that developed from the Big Bang and the giant stars to give the peculiar universal abundances of the elements and subsequently the limitations of the availability of elements in water on Earth and the inevitable selective binding of organic donors

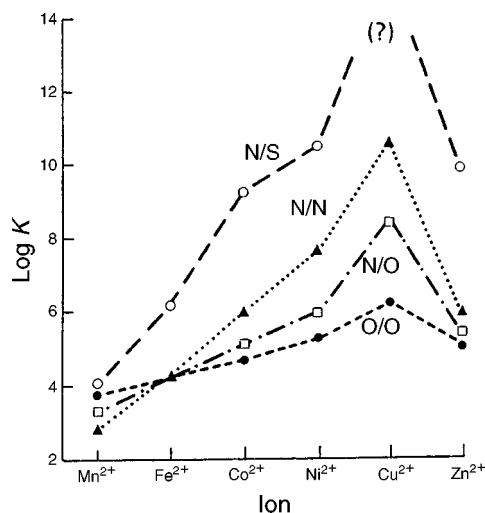


Figure 5. The variation of stability constants, K , for the complexes of M^{2+} ions of the Irving-Williams series. Examples of ligands are: O/O, oxalate; N/O, amino acids; N/N, ethylenediamine; and N/S, cysteine. The binding constants for ATP^{4-} are close to 10^4 M^{-1} for all the metal ions. In a cell at $\text{pH} = 7$ the effective constants must be used but they fall in the same orders.

to metal ions that are the decisive factors at the beginnings of life. It is these conditions that had to become codified in DNA, necessary for reproduction, and hence long-term survival. The variables, availability and binding strengths, are not biologically controllable. Hence the use of simple physical–chemical data in Figure 5 are justifiable in the discussion of early life. (This approach is however quite unrealistic for nonmetal elements). It may be useful to refer to simple class experiments to demonstrate the nature of availability, solubility, and complex formation. The tables of qualitative analysis (13), unfortunately discarded today, are a wonderful teaching aid for the chemistry of cells and the environment. For example those who are familiar with these analytical procedures for the separation of one metal element from all others know that, so as to increase selective reaction of a given reagent against competition by other metal ions, it is preferable to limit the concentration of the reagent through controlled addition or to use masking agents for the removal of competing ions.]

In cells there must be control over the ligand, here protein, concentration to manage selectively metal ion concentrations as in analytical chemistry. The cell control is exerted by feedback to the DNA expression system such that the free level of each metal ion is related to the production of its binding proteins via transcription factors. These factors are already well recognized in primitive anaerobic cells especially for dominant Fe^{2+} and Mg^{2+} either acting directly at DNA, for example, formate nitrate reductase (FNR) for Fe^{2+} (and note that this protein has an Fe–S cluster), or via controlled phosphorylation of transcription factors, via Mg^{2+} activation of kinases (and note that Mg^{2+} is bound by O-donors). We observe that such transcriptional control of the appropriate proteins is also related to binding constants of about 10^3 M^{-1} for Mg^{2+} and 10^7 M^{-1} for Fe^{2+} matching the free cell levels (Figure 4). A similar series of feedbacks acting on uptake and rejection pumps restrains element entry into and exit from cells. Note how the feedback connects the genome, the proteome and the metallome at fixed free ion and protein ligand concentrations but that it is also connected to the environment. There are parallel controls over “equilibrated” concentrations of free Na^+ , K^+ , Cl^- , and Ca^{2+} , as well as over nonmetal substrate and coenzyme concentrations, the metabolome (8, 9). These feedback–feedforward reaction systems provided the most primitive messengers of organisms and maintain homeostasis. We note again the *quantitative* integration of a trinity of code: code product, proteins, and the environment content of elements in a system (1, 14, 15). The most revealing facts are that the *concentrations of the free metal ions in the cytoplasm* are closely fixed for all cells and that mostly these concentrations are quite closely restricted by the free element content of the early sea (Table 1 and Figure 4).

It is important to observe that in systems such as these, in which a metal ion exchanges rapidly and hence equilibrium is approached, the functions of all of its proteins depend on the property that all the different metal complexes of one metal ion have approximately the same binding constant. Examples given so far in the cytoplasm of all cells are the binding constants for: Na^+ , 10^2 M^{-1} ; K^+ , 10^1 M^{-1} ; Mg^{2+} , 10^3 M^{-1} ; Fe^{2+} , 10^7 M^{-1} ; and Ca^{2+} , 10^7 M^{-1} (1). There are closely fixed values for Cl^- and HPO_4^{2-} , too. Of necessity, to

maintain a fixed metabolic organic reaction system, these equilibrium constants of the cytoplasm became effectively invariants of the cytoplasm for all time, and once again we stress that the values are dependent upon chemical necessity that had to become connected to the code, DNA.

Increasing the Variety of Coordination Complexes in Cells: Kinetic Traps

In the presence of sulfide some uptake of Co, Ni, and Zn, but not Cu, into early anaerobic organisms was possible owing to their coordination in N- and S-donor complexes (Figures 4 and 5), but it is difficult to see how much uptake or selectivity could have been achieved in such simple complexes. Later greater use of these elements arose from the separation of their functions in *kinetic traps* using ring chelates all derived from uroporphyrin (Figure 6), namely, iron in porphyrins, magnesium in chlorophylls, cobalt in vitamin B_{12} , and nickel in F-430. Trapping elements in this selective way avoids exchange and competition between them for organic centers and with free Mg^{2+} , Fe^{2+} , and Zn^{2+} . Not only did this allow use of a number of metal compounds behaving differently from the corresponding free ions, generating new concentration variables, but it gave rise to new functions such as photosynthesis and new sources of energy including the later use of free oxygen (Table 2). Further examples of this protected kinetic trapping are found in the uptake of molybdenum and tungsten into the pterin thiolene coenzymes and of molybdenum or vanadium in the nitrogenase FeMoco coenzyme. As stated in all probability Fe and V were used in nitrogenase before Mo, which was less available in the early sulfide rich environment (5, 11, 12).

While the free element concentration of the kinetically trapped elements in ring chelates is not directly linked to their complexes, that is, in complex chelates derived from uroporphyrin or from pterin-dithiolenes, the concentration of the chelates must be fixed in homeostasis. Hence we find, for example, that free heme and chlorophyll production is controlled to match the synthesis of their respective binding proteins. The *kinetic* connection of these controls to those of the free elements is only known in outline as yet, but is essential knowledge since synthesis of all the chelating agents is linked back to the free elements at the gene level.

Turning to the external side of the cell membrane the fact that Mn^{2+} was readily absorbed onto the outer surfaces of cells, which could capture light and that the energy of light is sufficient to disproportionate water via MnO^{2+} formation, may well have led to the possibility of the creation of O_2 from H_2O . At the same time the reaction gave a more abundant source of hydrogen for reduction in the cell than the use of H_2S . The incorporation of the activity into the whole of the cell and then subsequent modification of the system eventually through a code are further steps. This development of Mn chemistry, unlike that derived from uroporphyrin in *kinetically* separated chemistry in the cytoplasm (Figure 6), is an example of the use of separation as a result of spatial division by a membrane. It is the development of spatial separations of further new chemistry that drove evolution, but it is necessary to see that it could only occur with changes in the environment, all dependent ultimately on the coming of O_2 (16).

We can now proceed to the evolution of compartmentalized cells (Figure 7), noting that only in compartments are large changes allowed in more oxidative chemistry (including that of novel metallomes) since the central cytoplasmic activity is fixed of necessity by the requirement for a unique *reductive* production of nucleotides, proteins, lipids, and saccharides in all cells of all species. The development of compartments is central to an understanding of evolution but it is driven by chemical pressures and advantages provided by the environment.

O₂ and Evolution: Compartments and Oxidation

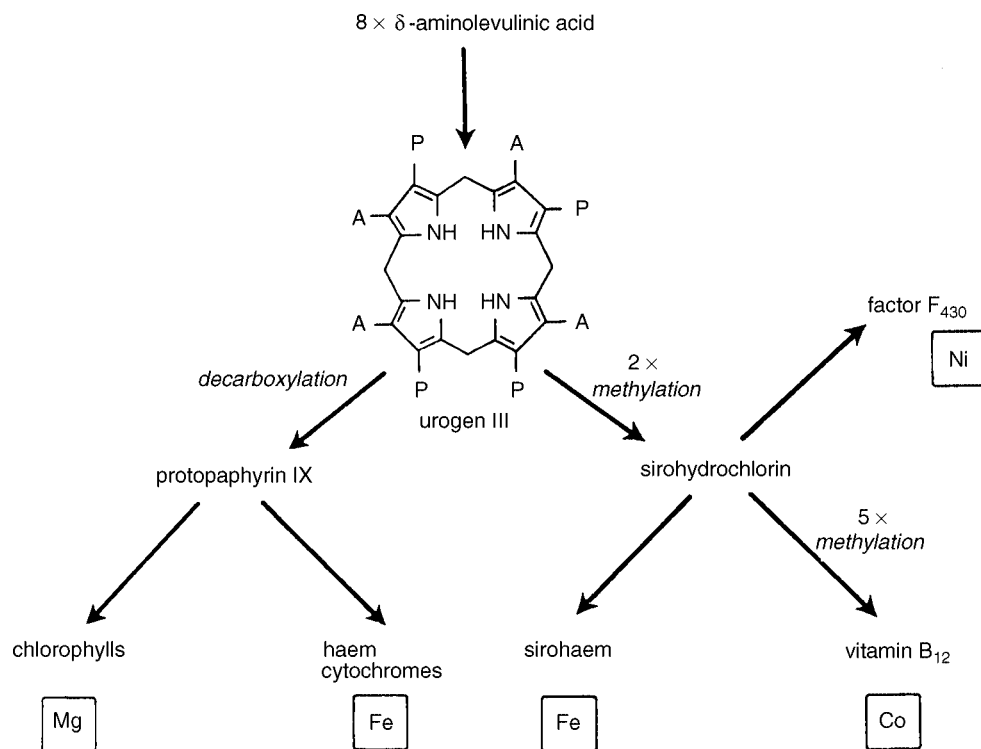
Oxygen in the atmosphere produced, consecutively, a range of novel chemicals (Figure 1). The only possible development of prokaryote cells in which the new oxidized chemicals of the environment could be used, was the creation of a concentric membrane compartment, the periplasm. An alternative development was the creation of many internal compartments but this necessitated a change in membrane chemistry and in the size of the cell (Figure 7). The earliest changes towards eukaryotes are known to have involved the incorporation in membranes of cholesterol, a relatively easily obtained product of oxidation of squalene derivatives. This change and that of other oxidized lipids, together with the development of internal filaments, permitted the cell to have a large flexible membrane structure unsupported by walls and subsequently to incorporate or create new compartments, called vesicles or organelles (List 1 and Figure 7). The earliest redox changes of elements (Figure 1) were C to CO₂, N to N₂, S to SO₄²⁻ and Mo to MoO₄²⁻. They provided a new supply of stored energy, SO₄²⁻ versus S²⁻, as well as from O₂. Most of these changes occur in the periplasm of prokaryotes

or in compartments isolated from the cytoplasm in eukaryotes, for example, glycosylation of proteins and sulfation of saccharides.

There were also deleterious changes, for example, with increasing O₂ the free environmental iron fell to 10⁻¹⁷ Fe³⁺, which meant that cells had to devise means of scavenging for essential iron, which they did and do today in amazing ways (17). The modes of uptake and retention in different species is varied but all have the common result of generating < 10⁻⁷ M concentration of Fe²⁺ in the cytoplasm, which is the level in anaerobic prokaryotes. Here is a remarkable example of the importance of the fixed cytoplasmic free metallome, which in our opinion is of equal consequence to the use of DNA in the genome. All other free metal ions (Co²⁺, Ni²⁺, Cu²⁺, and Zn²⁺) were kept below ~10⁻¹⁰ M in the cytoplasm to protect Fe²⁺ and Mg²⁺ (we shall see later how this is managed) but vesicles could contain high free Zn²⁺ for example. The essential point is that all the development of eukaryotic cells was dependent on the changed environment, which was inevitable.

Further Development of the Eukaryote Cell: Messengers

We need to see that the development of a compartmented cell, while it provided space for reactions not readily acceptable in the cytoplasm, needed a message system coordinating the new activities with the coded cytoplasmic synthesis. Many of the vesicles with which we are concerned were effectively inside-out enclosures derived from the outer membrane by invagination. As a consequence, since their pumps acted to remove ions from the cytoplasm, the vesicles took up from this primary compartment those ions previously re-



jected by prokaryotes, for example, H^+ , Ca^{2+} , Na^+ , Cl^- , and Mn^{2+} , but rejected Mg^{2+} and K^+ . Looked at from an osmotic electrolyte point of view the outside of the cell and the vesicles contained large stores of ionic, potentially current-carrying, ions relative to the cytoplasm. It was this capacity for generating ionic current carriers inside cells that was brought into use in signaling in the eukaryotes (10) so as to provide information about the compartments and even the environment to the cytoplasm and the DNA. Especially Ca^{2+} , through its unique binding chemistry (19) and strong rejection from the cytoplasm, became used for this signaling in single cell eukaryotes. It could only be used in short-lived pulses from outside or vesicles to the cytoplasm since Ca^{2+} is poisonous there (19). Cells, therefore, developed fast cytoplasmic and vesicular receptors for the Ca^{2+} pulse generated by outside or internal events and fast rejection of Ca^{2+} back to the vesicles and the external solutions. These receptors in the cytoplasm initiated responses to Ca^{2+} pulses especially in the primitive ATP- Mg^{2+} units (kinases) controlling mechanical and chemical activity down to the level of extra energy production for action. Immediately it will be seen that a fixed calcium concentration relationship between vesicles ($10^{-3} M$) and cytoplasm ($10^{-7} M$) could also be used to form shaped shells from $CaCO_3$, as seen in coccoliths. Other vesicles could store poisons or enzymes for digestion to be released externally on receipt of an appropriate Ca^{2+} signal. When mitochondria and chloroplasts (prokaryote derived organelles or vesicles) were incorporated in eukaryote cells (18) they too responded to Ca^{2+} messages that coordinated energy production to cytoplasmic demand. Thus the whole eukaryote cell became crisscrossed by a network of Ca^{2+} ion communication (Figure 8). This network is based on fixed values of the binding constant, $10^6 M^{-1}$, for all the protein complexes formed when Ca^{2+} invades the cell. The new proteins involved, calmodulins, annexins, S-100, and C-domains, are only found in eukaryotes. Note they use O-donor centers of large cavity size to avoid binding by any transition metal ion or even the small Mg^{2+} ion (19).

List 1. Novel Features of the Basic Eukaryote Cell

1. New membranes due to inclusion of cholesterol produced by oxidation
2. A zone, protected from oxidation, for the DNA code and its transformation in a nucleus
3. Production or incorporation of isolated compartments for:
 - (a) Oxygen production in chloroplasts
 - (b) Oxidation of small organic substrates in mitochondria
 - (c) Peroxide chemistry in peroxizome vesicles
 - (d) Export of poisons stored in vesicles
 - (e) Retainment of calcium ions in vesicles for message transmission
 - (f) Acidic hydrolysis in vacuoles or vesicles for digestion
 - (g) Glycosylation and sulfation in Golgi vesicles
4. Exocytosis and endocytosis of vesicles or large units
5. Increased use of zinc in the cytoplasm
6. Novel uses of several metals in oxidation or reduction reactions

NOTE: See Figure 7 (18).

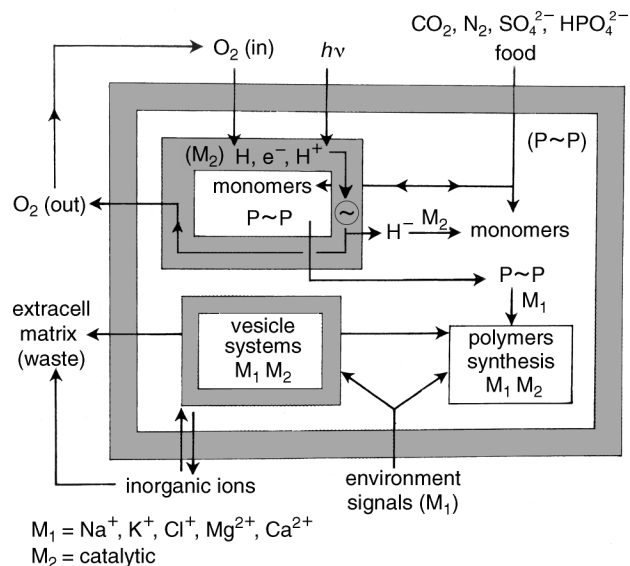


Figure 7. An outline plot of a eukaryote cell showing many compartments including vesicles and organelles (mitochondria and chloroplasts) top left. Note the roles of metal ions.

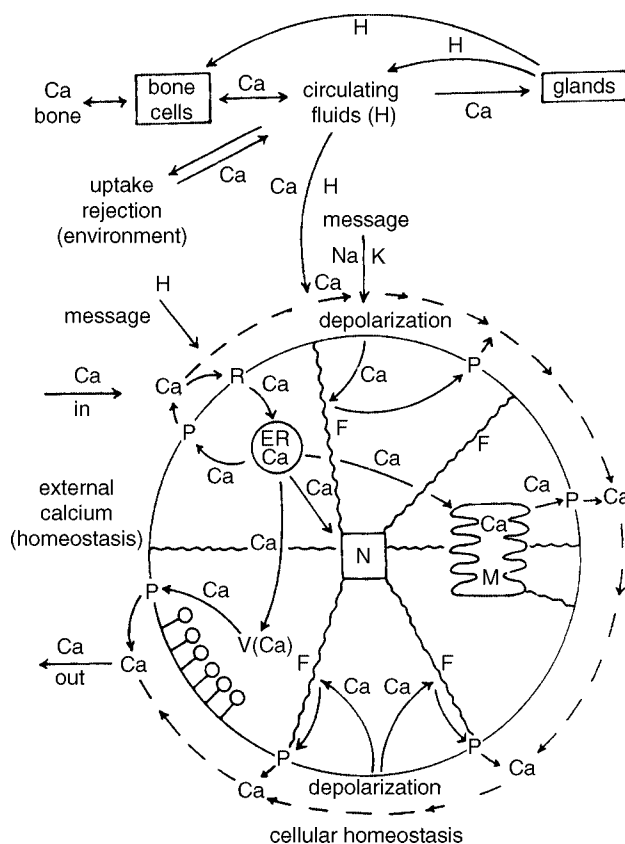


Figure 8. A schematic of the complicated network of Ca^{2+} interactions in an animal: H, hormone; P, pump; ER, endoplasmic reticulum; N, nucleus; M, mitochondria; F, filament; V, vesicle. The calcium ion has carriers (chaperones) and buffers in the cell, apart from triggering proteins of many varieties (19).

Another major communication network appeared in eukaryotes based on zinc ions. Zinc became available as oxygen oxidized its sulfide. (Note that Ni and Co were also released but they were separately held either in ring chelates or kept out of cells.) Some of the new zinc proteins—zinc fingers, which exchanged zinc—bound to DNA and coordinated novel activities linked to new oxidized organic messengers, such as sterols, related to cholesterol. Note that zinc in low concentration is not harmful to the cytoplasm since it has no redox chemistry.

The development of these and all other units of organization has to be clearly seen, therefore, as involving three inevitably interwoven steps: the development of (i) physical compartments, (ii) filamentous internal structures, and (iii) new messenger systems.

[It might be thought that mitochondria and chloroplasts, which use or generate O_2 , are examples of high redox potential chemistry in organelles that are derived from prokaryote cells (18). In fact the cytoplasm of these organelles has a reductive chemistry, obvious in the case of chloroplasts, while production of O_2 or its utilization occurs on the outside of the cytoplasmic membranes, that is on the periplasmic side].

Protection of eukaryotes and indeed of aerobic prokaryotes was also required from O_2 in the cytoplasm, especially after its partial reduction to O_2^- and H_2O_2 or other peroxides. This was provided in part in prokaryotes by the use of iron and manganese superoxide dismutases and heme catalase, and in part by the newly possible redox chemistry of selenium. Note that the earliest known protection in sulfate bacteria also uses a ferredoxin–NADH reductase but the last superoxide dismutases to be developed use Zn and Cu but only in eukaryotes.

Increasing Dioxygen in the Environment

As the concentrations of O_2 rose further, zinc increasingly and finally copper were released from their sulfides. At the same time higher redox potential chemistry, such as that of H_2O_2 and NO, arose. It is not until this last stage that plant and animal multicellular organisms appeared about one to two billion years ago (Figure 2 and List 2). The reason lies in the requirement of *multicellular systems for external filamentous lattices of relative fixed structure*, which can only be obtained by external oxidative crosslinking, and for a further novel system of external messengers now linking cells together in organs. The requirement is based on higher redox chem-

List 2. Novel Features of Multicellular Organisms

1. Differentiated cells later in organs
2. Controlled extracellular fluids, plasma, and cerebrospinal fluid
3. Connective tissue strengthened by oxidative cross-linking
4. Extensive use of oxidized small organic molecules and peptides as messengers
5. Oxidative chemistry of amino acids in proteins and peptides
6. Oxidative enzymes based on copper or heme iron outside cells and in vesicles for connective tissue cross-linking and the production of messenger organic molecules
7. Use of oxidative chemistry of halogens, e.g., iodine in thyroxine
8. Decreased dependence on nickel and cobalt chemistry, more useful in reductions
9. Sodium and potassium messages (in nerves)
10. Increased use of increasingly available zinc in hydrolysis, control and message systems
11. Use of selenium in peroxidases
12. Loss of independence from lower forms of life
13. Increase of use of mineralization, e.g., in bone

NOTE: Refs 8, 9.

istry. The external crosslinking chemistry was first managed in plants using H_2O_2 generated by heme plus external rejected Mn enzymes, peroxidases, and ligninases to give lignins, which stabilize dead rigid external saccharide structures as seen in wood. While a plant grows using this free-radical oxidizing system, this is not possible for animals. Animal growth requires *safe* chemistry for crosslinking to establish living, adjustable multicellular structures to which *unsafe*, free radical, chemistry could cause disease, such as cancer. It is observed that in animals it is copper enzymes that are used in external *oxidases*. These oxidases avoid H_2O_2 production. To allow growth there are also external zinc enzymes to breakdown these external structures (collagenases, elastases, etc.) alternating with their construction. It was only through the use of zinc and copper that the development of animals became possible. (Note the limitation on the external use of iron owing to its precipitation as ferric hydroxide).

Simultaneously with these developments there was required signaling between cells or groups of cells, organs, to control responses and growth. Here the new messengers, hormones and transmitters, are often synthesized by oxidative

Table 4. Organic Messengers Produced by Oxidation

Messenger	Production	Reception	Destruction
NO	Arginine oxidation (heme)	G-protein	Heme enzyme?
Sterols	Cholesterol oxidation (heme)	Zn-fingers	Heme enzyme
Amidated peptides	Cu oxidases	(Ca^{2+} release)	Zn peptidases
Adrenaline	Fe–Cu oxidase	(Ca^{2+} release)	Cu enzyme?
δ -OH Tryptamine	Fe–Cu oxidase	(Ca^{2+} release)	Cu enzyme?
Thyroxine	Heme (Fe) peroxidase	Zn finger? ^a	Se enzyme
Retinoic acid	Retinol (vitamin A) oxidase	Zn finger? ^a	Heme enzyme

^aIn the nuclear receptor super family of transcriptional receptors.

mechanisms. Again we observe the overwhelming importance in this high redox potential chemistry of iron (heme) and copper in oxidative enzymes in the production, reception, and destruction of a variety of oxidized organic messenger molecules (Table 4). Note that all the available inorganic ions that can bind to organic molecules and therefore act as messengers, Ca, Mg, and Fe, had already been employed, as had small *reduced* organic molecules, as messengers in the cytoplasmic homeostasis. Running in parallel with the appearance of the new messengers are the development of zinc proteins for the hydrolytic production and destruction of additional peptide hormones. In series with the oxidatively produced hormones (sterols, retinoic acid, thyroid hormones) we again find further use of zinc proteins, the so-called zinc fingers, as transcription factors. It was especially this new combination of uses of now available zinc, *outside* and *inside* cells, and copper together with the novel uses of heme and manganese enzymes, *outside* cells, plus the new organic messengers between cells, made by oxidative reactions, that allowed the explosive developments of living species about one billion years ago. We elaborate on these features in our books (1, 14, 15). (Note that oxidative organic nonmetal chemistry also developed during this period. For example selenium and iodine, especially in vesicles and outside the cytoplasm, were used to produce and modify thyroxines, and molybdenum was used in the oxidative production of, for example, the plant growth hormone, abscisic acid, by an aldehyde oxidase.)

Throughout this development of the *total* metallome, the free cytoplasmic metallome hardly changed: free Cu and Zn in the cytoplasm remained well below 10^{-11} M. Simultaneously, the use of nickel and cobalt, largely of value in reductive reactions at low potential, fell. Nickel is used mainly

in one enzyme, urease in higher organisms, while cobalt is found only in vitamin B₁₂ and even this vitamin is absent in higher plants. The introduction of molybdenum is kept to below 10^{-6} M MoO₄²⁻ in cells (5, 11).

We see that organization, in a chemical system, has to be based upon the simultaneous development of compartments, even in organs, and new chemistry to provide new functions, together with connecting filaments and new messengers. In effect this general trend of evolution with increasing oxidation of the environment is inevitable though of unknowable timing. A major consideration in evolution is then the way in which the uptake and utilization of elements from a changing environment can enable development (15; Table 5).

As complexity in an organism increases, it becomes more efficient not to be competitive but to trade between functional diversity in different organisms, effectively an increased use of compartments. In symbiosis the different organisms exchange materials. Thus we see mutually dependent ecosystems of organisms. A particular case is that of the appearance of mobile animals dependent on foraging plants for essential chemicals. The gain in the foraging capacity with sensing equipment is matched by the loss of the ability to take in elements, to absorb energy from light, and to synthesize essential coenzymes, amino acids, fats, and other compounds. A further case is the need of plants for bacteria (N₂ fixation) and fungal hyphae to collect minerals. (The parallel with the development of man's industry where mutually dependent units make the whole system efficient as, say, in the manufacture of automobiles is clear. As in this example, central control of organization remains however an overriding objective).

Table 5. Involvement of Elements in Homeostasis during Evolution

Primitive Anaerobic Prokaryotes	Early (Anaerobic) Single Cell Eukaryotes	Later (Single Cell) and Multicellular Eukaryotes (Aerobic)
H, C, N, O, P, Se (hydride transfer) substrates and polymers		Oxidized chemical forms Se peroxidase
H ⁺ , Na ⁺ , Mg ²⁺ , Cl ⁻ , K ⁺ , Ca ²⁺ exchangers and ATP-ases		Outer filaments and signaling Na ⁺ /K ⁺ between cells, Na ⁺ ,K ⁺ -ATP-ase organic hormones, iodine hormones
Ca ²⁺ structural	Ca ²⁺ vesicles and inner filaments and signaling via calmodulin	Annexin, S-100, and other Ca-binding proteins
H ⁺ , P, S, Fe signals	P elaborated	Further elaboration
W enzymes	Some Mo enzymes	Only Mo enzymes
Mn, Fe, Mo, Se low redox potential enzymes	(O ₂ release from Mn enzyme photosystem)	High redox potential enzymes
Ni enzymes (H ₂ , CO), Ni (urease), Co (B ₁₂)		Plants only, animals only
(Zn enzymes)? structural?	(?) Zn enzymes increase	Zn enzymes in vesicles, Zn signaling (DNA), Cu enzymes

NOTE: See Chapter 20 of ref 1.

Simultaneously with development of these devices for making viable multicellular systems arose the need for further protection. Once again we see how one large part of protection in eukaryotes is controlled by heme, copper and zinc proteins as catalysts utilizing the chemistry of oxygen, nitric oxide, and halides. To remove the basic dangerous products of O_2 reduction there are the catalases (heme) in vesicles and superoxide reductase dismutases (but they are now Cu, Zn proteins in the cytoplasm of eukaryotes). These agents, O_2^- and H_2O_2 , were also turned into aggressive chemicals for the destruction of other invading organisms, but only with unavoidable risk. Plants use H_2O_2 extensively with many *external* heme peroxidases, while myeloperoxidase *in vesicles* is a major animal protective agent of the immune system for the generation from halides of halogenated agents, for example, ClO^- , for cellular (bacterial) destruction. (Oxidative chemistry of halides was a late development since it required high redox potentials.) Of course, plants use peroxidases to ward off invaders by the simple device of closing off damaged zones by modifying dead connective tissue as in browning and crown-gall production, but this permanent retention of protective tissue, as is seen also in the bark of trees, cannot be risked by animals that have circulating cells. Thus animals developed circulating cells for protection of space external to organs. We must also stress again how the evolution of the chemistry of the elements in organisms is following that of the oxidation of the environment, but it is now the bound metallome especially in vesicles and specialized cells that evolved with a communication system now based on flow within extracellular space.

In the case of animals a further system of messages, a nerve system, was required to coordinate sensing and muscular activity to aid scavenging. This final step in evolution (of biological inorganic chemistry) is to be seen in the vastly increased functional value of sodium, potassium, and chloride in the nerves of animals. Here use is made of their ionic gradients, produced initially in the management of osmotic and electrical balance in the most primitive cells (see above), to function in *fast* electrolytic messages—the conduction of current along nerve cells. Only Na^+ , K^+ , and Cl^- are sufficiently abundant and free as ions to generate this conductivity. For this activity a special Na,K ATPase was synthesized. The initial electrolytic stimulation connects to slower Ca^{2+} messages and is usually followed by even slower organic messenger release as a result of a pulsed calcium invasion at cell termini. The nervous system construction of growing cylindrical cells within connective tissue was not possible, of course, until this tissue was laid down with the help of zinc and copper enzymes. In order to coordinate the nerves of the motility organs, muscles, and the sensing organs, the nervous system evolved an organizing center, the brain. It is the brain that allows collective organized activity inside one form of life and also in organization into societies of individuals. Finally, the brain was placed in a special extracellular (cerebrospinal) fluid. Again special chemistry arises together with special compartmentalization to produce new emergent properties. It is the further advance of this fast system of communication in the brain linked to the organic messengers in multicellular species that led to man and his dominance.

Mankind

The last possible development in this system of increasing use of the chemical elements and of compartments is that of man's chemical industry. This is the ultimate illustration of the independence of the inorganic environmental variable from that of the two variables of the organic chemistry of cells, the genome, and the proteome. Man uses all the chemical elements, not just the twenty under biological control, and all of space in compartments. The compartments are organized by connected transport and message systems as in all organisms. We have no space to go into details but it is an exercise for students to see first how nonbiological organization parallels that internal to organisms and then to consider how mankind's activities can be managed so as to preserve the biological ecological system that has evolved.

Conclusion

The purpose of this somewhat simplified article (see our books, refs 1, 14, 15, for a more extensive discussion) is to show that the evolution of cellular structures and their

List 3. Levels of Self-Organization^a

- A. Prokaryotes (3.5×10^9 years ago)
 1. Single membrane containment
 2. Differential, inside-outside, distribution of elements
 3. Polymer-polymer synthesis and interactions
 4. Relatively simple messenger-based coordination
- B. Eukaryotes, single cells (2 to 2.5×10^9 years ago)^b
 1. Multiple membrane-limited compartments and later organelles
 2. Differential distribution of elements and large molecules in B1
 3. More complex polymer-polymer interactions including filaments and external mineralization, which generate very selective shapes
 4. Complicated messenger systems linking compartments and the environment, especially using calcium
- C. Multicellular organisms (about 1.0×10^9 years ago)^c
 5. Multiple cell-limited activities, organs
 6. Differential distribution of all chemicals in cells
 7. Increasing control over extracellular body fluids, filaments, and minerals
 8. Messenger systems linking cells (nerves) via transmitters, hormones, etc., organic in nature
 9. Central coordination (the brain) in more advanced animal organisms ($< 0.5 \times 10^9$ years ago)
 10. Symbiosis between A, B, and C

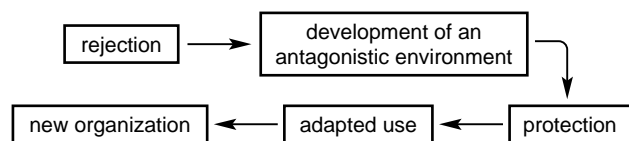
^aThe reader may wish to add other features to this list.

^bEukaryotes evolved from the archaebacteria plus organelles from eubacteria.

^cThe extra organization is now of eukaryotic cells, so we add to 1–4 in B.

chemical functions, as well as of molecular units associated with them, was dependent on and linked to environmental oxidative changes of the elements generated in part by living organisms in *an inevitable feedback succession, within an ecosystem* (20). The reductive chemistry of the cytoplasm and its rejection of some elements in primitive life was complemented by oxidative chemistry and the created gradients around the cells in added-on compartments and procedures in them and then in multicellular organisms. Thus the changes with time in living organisms marched in step with the inevitable earlier changes of external availability of elements and element oxidation states. The changes in energy capture also matched these environment changes. The efficiency of this capture was of necessity connected to the whole of an ecosystem of organisms to reduce local complexity. Exactly how the program machinery, the DNA of individual species, adapts to the environment is not discussed here. In effect we have left aside the whole problem of the need to develop genetic structures while we looked at the overall ecological states. Coexisting species may be seen as a selection of possibilities opportunely using roughly equally an environment at a given time. We cannot see a logic to the evolution of particular species, for example, cows, sheep, deer, and goats. The evolution of kingdoms is logical.

The realization that we are dealing with an overall system, internal and external to life, must lead to increased environmental awareness by mankind so that further evolution, whatever that may be, can be managed by rational consideration. The environment in an ecosystem includes the essential inorganic elements and the mutually interacting organisms, but inevitably man's products from new external organization (e.g., productive industrial activity) will become chemically coupled to evolving life. Interestingly, all the changes we have discussed follow the pattern of new element use



Here adapted use includes active metabolism, new energy and message activity, and new structure building, including compartments. The future will follow this pattern too.

Finally note again that evolution is described here in cooperative dynamic *systems*, not single molecular language associated with species (List 3). Thus we see evolution as changes in emergent systems, organisms plus the environment, not just in molecular mutational terms, since controls and resources must be added too. There is no easy correlation between one-by-one analysis of changes of molecular properties, no matter which molecules are chosen (DNA, RNA, or proteins), and changes of a system activity, which is part of an overall emergent developing property we call life.

Addendum

Since this paper was submitted a very interesting parallel account of chemical systems in organisms has ap-

peared in Sterner, R. W.; Elser, J. J. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*; Princeton University Press: Princeton, 2002. See also *New Scientist* 2003, January 18, 12–13 for comments on the ideas expressed ref 20 and references therein.

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