Acta Biochimica Polonica

Vol. 51 No. 2/2004

281–298

QUARTERLY

Review

Signalling: basics and evolution★

Robert J.P. Williams✉

Inorganic Chemistry Laboratory, University of Oxford, Oxford, U.K.

Received: 17 April, 2004; accepted: 19 April, 2004

Key words: signalling, metal ions, substrates, pumps, receptors, donors

Signalling concerns the transfer of information from one body, a source, to another, a receiver in order to stimulate activity. The problem arises with the word information. It is defined as what is transferred in a sequence of things, say between people, e.g. words or signs. The idea of signalling between people is then obvious but it is not clear in cell biology. Information transfer, signalling, is required for the organisation of all cellular activity but we must ask what is transferred and how is it transmitted and received? Sometimes it is assumed that all information, i.e. organisation in a cell, is represented in the DNA sequence. This is incorrect. We shall show that the environment is a second source of information concerning material and energy. The receiving party from both DNA and the environment is general metabolism. The metabolism then signals back and sends information to both DNA and uptake from the environment. Even then energy is needed with machinery to send out all signals. This paper examines the way signalling evolved from prokaryotes through to man. In this process the environmental information received increased to the extent that finally the brain is a phenotypic as much as a genotypic organ within a whole organism. By phenotypic we mean it is organised by and interactive with information from the environment.

I have been asked to discuss signalling. It may be thought that the word has an obvious meaning but I shall show that this is not the case. Signalling is defined as an activity that sends out information from one party to another with the intention of bringing about a certain consequence. It is not the consequence itself. That lies in the interpretation

★Presented as invited lecture at the 29th Congress of the Federation of European Biochemical Societies, Warsaw, Poland, 26 June–1 July 2004.

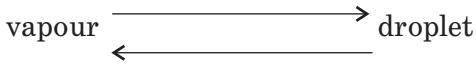
✉Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QR, U.K.; tel.: 44 (0) 1865 272 621; fax: 44 (0) 1865 272 690; e-mail: bob.williams@chemistry.oxford.ac.uk

and activation on receipt of the message. Now the difficulty here is that "information" does not have a very obvious meaning. If we define information as that which is transferred in a sequence of things it is easily understood in the messages between two people because they have language, for example, in common, and they have equipment with an energy supply to send out, to receive and so act on these messages. What is the equivalent structure in lower organisms?

If we start again from the idea that information is that which is conveyed by a sequence of things then we see that for there to be such conveyance, there has to be an energised source, a sending out of energy or material (a signal), and a receiver which is affected by the energy or material so that it responds, but the response and the signal are not the same. Notice in particular that the signal is in a sequence and therefore has a directional character on reading. Clearly conveyance is improved if it is guided from source to receiver.

Now we have to be careful with the above descriptions relating to signalling since we must distinguish a system which is "formed" by the bringing together of various flowing parts in a construction and an "informed" system which has *in addition* a formative message by which one formed system affects another. First we describe some abiotic systems so as to clarify definitions.

Consider the sun as a source of radiation and Earth as creating a gravitational field. The sun heats Earth's surface generating a temperature gradient. These two sources convey energy to the sea in gradients and affect the behaviour of water so that it rises as vapour and falls after condensation. There is an interesting intermediate steady state in which the water forms clouds. The water in a cloud is also affected by the horizontal movement of the air, which again arises from the sun's radiation through the creation of differential pressures in different places. These movements are called winds and they convey directional momentum to the water vapour both as vapour and droplets. Following our definition the resultant clouds, which form shapes and move in a regular way, are not just created by these sources but they confine them forcing a given behaviour as part of a "formed system". The source of form is the sun and Earth, which convey energy in a continuous sequence. However, the energy is received by water in both vapour and liquid droplets, in a particular directed way so that together they are affected so as to form the clouds. We could say the sun and Earth transmit to the water. All three have in common a quality, energies, which the first two send out and the third receives. The water responds by forming clouds. To this system consider the addition through some action of small crystals, even dust. These particles have a surface and the surface has a structure. Water molecules in the atmosphere bind to the surface to form droplets and then can be released so that a cloud formation is engendered. The crystal surface acts as a source of information aiding the process but it is not a part of the system itself. It is not essential once the result is achieved. The system does not involve physical or chemical change in the particles. Note the cloud is eventually the result of a cyclic steady state no matter whether dust is present or not



where the path forward is different from the path back and the dust informs the path forward. The dust is from an outside source. We turn next to a chemical example.

Oxygen absorbs energy from the sun to give ozone. Both oxygen and ozone absorb gravitational energy conveyed by Earth. There is directional as well as energy content. Together O_2 and O_3 form the ozone layer at a given height above Earth. The donors of energy are the sun and Earth, the receiver is $O_2 + O_3$, energy is transferred and the oxygen responds to give a *shaped object* the steady state ozone

layer. The whole is a "formed" system. The chemical cycle is

$$3O_2 \rightleftharpoons 2O_3$$

The addition of catalysts to either process informs the system. The catalyst is from an outside source. Clearly a "formed" system can arise from one substance in a cycle but an "informed" system requires an additional substantial system to apply a thing, a signal, to the single substance system when it becomes "informed".

We shall give one further example. We shall see that the shape of a river is a consequence of water flow in an "formed system". The water, the receiver, has been energised from its origin in the sea and falling as rain on high ground due to gravity it flows down-hill again due to gravity. Hence the flow is picking up directional gravitational energy conveyed from Earth and heat, conveyed from the sun just like a cloud. It is a formed system. Now as it flows, the river follows contours of the land within banks, which act as directed repulsive physical barriers. Banks convey repulsive energy and rivers follow a meandering shape. The water is guided. Here a new repulsive energy has been conveyed to the flow by a barrier, which therefore forms the water flow. The bank is not part of the flowing system, which is energised water, but forms its course.

Only now are we ready to tackle formed and informed systems in a reproductive cell. We must define its form and information (signals) and the sources and receivers of that information. Clearly material from outside the cell membrane, and energy from the sun for example, are obviously formative and this material and energy act in many ways so that the mode of conveyance of flow is continuously changing in materials inside the cell. The materials form concentrations (gradients) which then inform one another. We treat form first.

(1) ATP is formed after potential energy, charge-gradients formed by external energy causing flow of electrons and protons, is applied in protein machines, to which we return. This is oxidative and photo-phosphorylation. The source of energy is the sun or external chemical energy, O_2 + sugars. (Compare the sun's effect on water.) Since ATP decays the ATP cycle becomes a formed steady state like a cloud.

(2) Uptake and rejection of elements at the cell membrane, a barrier, by pumps, worked by chemical energy transfer, e.g. ATP, form many internal concentrations and gradients of metal ions and small substrates. They too can decay or react further to give a formed steady state.

(3) Inside the barrier the elements taken up are metabolised by chemical sources of energy, for example ATP, using enzymes to give larger substrates and biopolymers. These form new gradients of flow in synthesis and degradation to give a further steady state.

(4) The proteins, enzymes, also make bases using basic environmental material and energy and these bases are polymerised to give RNA and DNA.

(5) The metabolism also makes saccharides and lipids which form walls and membranes.

The whole generates a "formed system" which has no well-defined content. To create a defined content, homeostasis, seen in any cell there are several extra reactions which inform all the activities.

(6) The concentration of ions and substrates in the cytoplasm feed back to the pumps to stop uptake at a certain value, so that their concentrations are in fixed steady states. *The pumps are informed by the very objects they pump by this feedback*. The ions and substrate concentrations are signals, see Fig. 1, recognised by binding constants.

(7) The proteins are synthesised and informed, under the influence of RNA, in sequences of amino acids. RNA is also synthesised and informed by the sequence of bases of DNA. DNA with energised machinery can

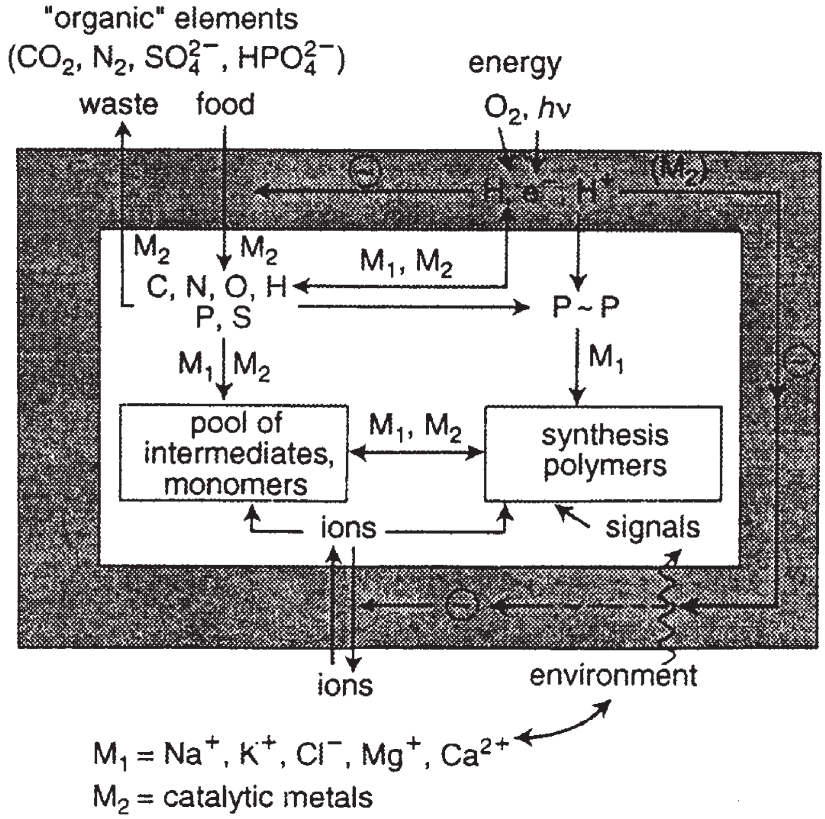


Figure 1. An outline diagram for the internal basic interactions in prokaryote cells.

There are no vesicles or organelles in prokaryotes so that they have only one containing membrane. The signalling is very strong internally but there is not a very strong response to the environment.

self reproduce and self inform. The sequences are determined by binding energies along the length of RNA and DNA and the knitting together in syntheses using ATP energy so that there is directed flow of connection of bases as of water in a river – the polymerisations have guided informed routes in sequences. (see the definition of information)

Note the every-increasing use of barriers as conveyors of information – extensive signalling.

(8) The particular amounts of DNA, RNA and proteins formed arise from the consequences of the concentration gradients of substrates and metal ions of various kinds in (3) and (4) which act through binding to transcription factors, proteins. The transcription factors thereby inform the DNA synthesis machine. The interaction of substrates and metal ions with the proteins in (1) pumps (2) machines (3) enzymes (4) enzymes (6) machines together with (8) proteins which bind RNA and DNA as transcription factors, means that the substrates and ion concentrations signal and act as information to them all. These interactions then form feed-back flowing conveyance loops to all activities. Information (signalling) is widespread throughout the cell creating holistic organisation.

(9) The energy carriers, NTP, also are allosteric effectors, informers, of much of metabolism acting on enzymes through binding constants and their concentration.

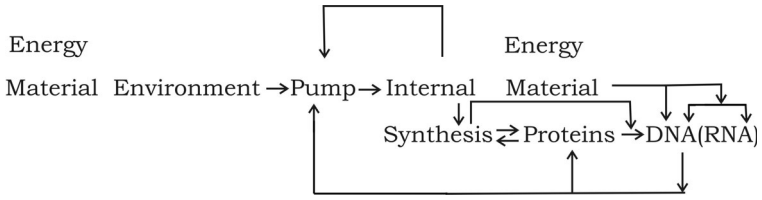
The whole is a back and forward interacting, reproducing set of activities where information is in energy and material transfer. Signalling is a complex concept in a cell and makes for an informed internal system of many separate pathways. In fact a system of many pathways cannot form a unity without

signalling between them. This initial evolution of signalling is as big a mystery as the formation of biopolymers on Earth.

Let us restate the position of internal signalling. It is often stated that DNA carries the information of the cell but this is true only for us to the degree that we have learnt to read the DNA directionally in terms of amino-acids generating proteins with known activities. For the DNA to carry information in a cell requires reading machines and energy which create the RNA and then proteins using other machines, ribosomes, from energy and basic chemicals pre-synthesised as bases and amino acids. Some of these proteins are transcription factors, which inform the DNA machine as to when it should act. This information is in part about the concentrations of the very substrates used in metabolic paths and in part about the supply of food, minerals and energy, obtainable from outside sources, the environment. The outside environment content supplies metabolism but also by binding informs both the membrane and the DNA of its quantitative content. The supply from outside is assisted by membrane pumps, which use energy but also are informed about inside concentrations by feedback by binding constants. Hence there is an interactive signalling, passage of information, which creates a cell.

Both chemicals and energy are conveyed from source to receiver to generate a signalling activity as well as being part of the activity. Thus ions, substrates, proteins, DNA (RNA) have a control activity as well as participating in metabolic events. In essence the starting point of signals is two-fold, the environment and DNA, which become interactive as the intermediate products in the cytoplasmic reactions feed back and forward to both the environment *via* the membrane and the DNA. It may seem strange but the very objects that pick up the signals become signals to their own metabolism and parts of the machinery at all levels.

I shall assume from here on that the above is an agreed view of formation, signalling and information. We need then to specify more clearly the materials involved in signalling in different living systems but we shall stop short of considering mankind except very briefly. We start from signalling in the simplest cell – a primitive bacterium, Fig. 1. Signalling is now the main means to internal cooperation of activity in its organised system. We distinguish here an ordered from an organised system since ordered systems are fixed and do not need signals while organised systems are dynamic in which material and energy flow. When several separate flows are needed to produce a unified activity then each flow has to be constrained by signalling. The picture of a bacterial cell is given in Fig. 1 with flows of energy and material. As stated above the number of signals in the system is huge. We list some in Table 1. Every unit signals to more than one other in the interior of the cell so as to make it self-regulating and reproducing as listed above. The informed system includes the environment, the source of initial gradients, since binding to the surface



activates the uptake. The surfaces sense gradients, see above. We must ask now how informed is a bacterial cell about its environment. It is this information transfer together with that transferred from DNA that has evolved greatly in higher organisms and is usually placed under signalling.

The metabolism of bacteria is adjusted to the supply of all nutrients, which it obtains from the environment. These include sources

of food, organic and inorganic. The outer membrane or cytoplasm has sensors and pumps for certain organic molecules and inorganic ions and they relay information to the DNA, using c-AMP for example as a triggered intermediate messenger, a signal, to transcription factors. In response the cells produce uptake or rejecting systems from DNA, e.g. the pump systems, but this type of response is only to molecules which have been met previously. Transcription factors also sense directly small molecules such as CO, NO and O_2 which penetrate the membrane. The DNA machinery responds by producing proteins relevant to these metabolites. Foreign molecules are not recognised until mutations create new proteins. The bacteria recognise particular mineral elements in the environment so as to obtain the essential uptake of Fe, Mg, Ni and so on, the concentrations of which connect directly to the DNA through transcription factors but are constrained by feedback to uptake. They even develop capture systems for Fe in response to a signal of iron deficiency. One could say bacteria have a limited informed *sense of smell*, chemical recognition, but a poor sense of their physical surrounds except for some species, which have magnetic/gravitational sensors. They are chemitactic in response to signals. Later light became used directly as a source of energy and as such it became part of a formed system much as it did in the ozone layer. The impact of light provides energy and substrates but the energy and substrates also signal to the transcription apparatus, dark/light switch, initiating required protein formation. So that light intensity, like environmental chemicals, directly informs the system it formed.

Table 1. Examples of elements used in early controls

Element	Control (mode of use)
H	NADH (NADPH), mobile coenzymes
e/H^+	Thiolate 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_2O levels or osmotic pressure)
Fe (haem)	Control in slow exchange

Let us look in more detail at some specific examples of *internal* bacterial signalling. There are mobile coenzymes and substrates, which carry H, C, N, S, P fragments but also act as allosteric signalling switches of activities. For example, NTP is a condensation reagent and before moving on notice that it is Mg · NTP which is active. Thus Mg^{2+} concentration must be controlled in an active cell. In fact most cells have both free Mg^{2+} and free ATP close to $10^{-3.5}$ M and bound Mg · ATP at 10^{-3} M. Mg^{2+} can only be pumped into or out of a cell by ATP (or a proton gradient) so that every cell depends on the informed gradient of Mg^{2+} between outside and inside. The cell has these two basic gradients which then in-

form it. Mg^{2+} is one type of messenger and ATP is both a messenger, or related to one, inside a cell, and a metabolite (MgATP), Mg^{2+} and ATP are internal signalling units. Note binding constants to *all exchanging (message) sites* must be fixed at about $K = 10^{3.5}\ M^{-1}$ so that the two Mg^{2+} and ATP coordinate a vast number of activities so as to help to establish homeostasis, Fig. 2.

concentration, about 10^{-7} M, can act also as a signalling link between metabolic paths so long as its concentration is fixed and the binding constants *to all the enzymes,* pumps and transcription factors are fixed and similar at $10^{7}\ M^{-1}$. This homeostatic condition is maintained and regulated by intake mechanisms, pumps, and by their synthesis and of the iron enzymes using the transcription factors FNR

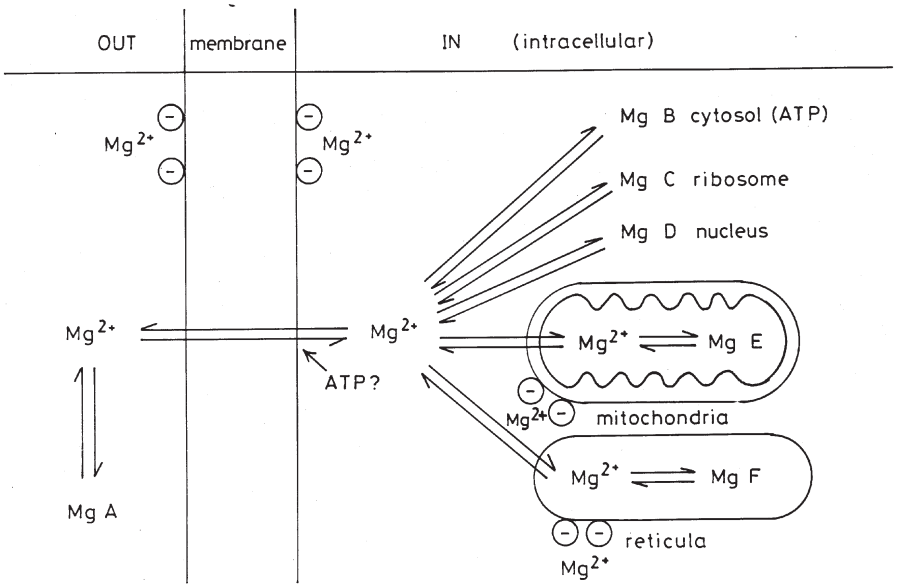


Figure 2. The multitude of interactions of closely equal strength of the Mg^{2+} ion with pumps, DNA, RNA, ATP, enzymes etc.

Mg^{2+} then acts as a coordinating signal in all cells. Other ions which work in a similar signalling way are Fe^{2+} and later in evolution Zn^{2+}. Contrast the pulsed actions of Ca^{2+}, K^{+}, Na^{+} and Cl^{-}. (N.B. The diagram is for a eukaryote cell.)

Now Mg·NTP reactions are concerned with acid/base changes but there are also necessary oxidation/reduction reactions to produce the correct degree of reduction of C, N, S and Se compounds for balanced combination leading together with acid/base reactions to fats, saccharides, nucleotides and proteins. Looking at the need for basic oxidation/reduction metabolism using environmental N_2, CO_2 and SO_4^{2-} we find that the ferrous iron, Fe^{2+}, is nearly always involved as an electron carrier in the reactions and more directly in enzymes. Moreover, this cation exchanges quite rapidly from binding sites so that its and FUR, which bind iron. All feed-back is controlled by the binding constant $10^{7}\ M^{-1}$. Thus Fe^{2+} is involved in complicated extensive signalling which is self-regulated. We conclude from these and other observations that of necessity and regulated by equilibria there are coordinating signalling units, which are held, fixed in a given state of a system, the metallome. Ions involved are at least K^{+}, Na^{+}, Cl^{-}, Mg^{2+}, Mn^{2+}, Fe^{2+} and later Zn^{2+}, Fig. 3, together with the mobile coenzymes including ATP and some substrates. With DNA/RNA, they generate an informed system internally linked to the outside environment.

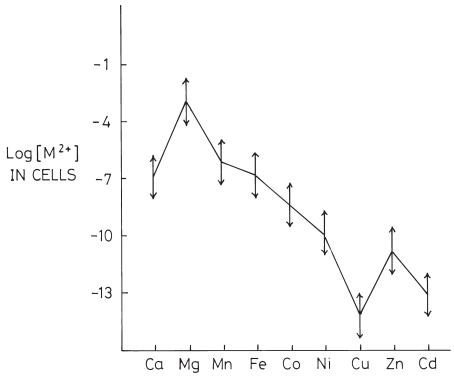


Figure 3. A plot of the logarithm of the free metal ion concentration in the cytoplasm probably for all cells.

The downward arrows indicate that the value may have been somewhat overestimated. There is considerable uncertainty about the smallest values.

In effect a bacterial cell has a tight feed-back relationship between in-take rates, metabolic rates and the supply of proteins. The equivalent statement is that the metabolic system is informed by the environment and the DNA, while the metabolic system informs access of the environment to the cell and expression of the DNA. There is a network of exchange of information, signalling, concerning the controlled conditions of energy and material flow into and out of the cell. To understand the cell in a steady state we need the concentration terms of the metabolome, the proteome and the metallome as well as of the DNA sequence, the genome.

Now we must note that the bacterial cell is only informed directly about very few parameters in the environment such as the concentrations of Na^+, K^+, Cl^- (their pumping creates electrostatic fields), Mg^{2+}, Mn^{2+}, Fe^{2+} (their internal levels control metabolism), sources of molecular C, N, S and P, substrates and coenzymes (for metabolism). This information is about basic nutrients and is like a sense of smell. We need to explore further the nature of the so-called "information" transfer to the cell and the link to DNA through signalling so that we can appreciate its role in later evolution. The slow development of what we recognise as senses, is central to evolution for it increasingly introduces *via* signals the environment into the state of an organism.

THE SINGLE CELL EUKARYOTES

We see that signalling in bacteria is very complicated but their sensing of the environment is very incomplete. In effect they have only a sense of smell, incoming chemical and energy analysis. The coming of oxygen in the atmosphere changed the environment and produced much larger cells, the eukaryotes, with longer life cycles. These organisms are capable of feeding off bacteria, even of incorporating them in the cell activity as organelles and they developed, for good reason, many vesicular compartments, Fig. 4, see references. A great advance in them was of necessity signalling to organise all the compartments. They had to maintain of course the internal signalling of the cytoplasm and the same sensing of the environment as bacteria but there was now required back and forth communication with the compartments and organelles. Because of their longer life-time and ability to feed off large particles including bacteria, it was a great advantage for them too to be able to recognise additional features of their environment and to signal this recognition to the cytoplasmic, organelle and vesicle activities and DNA. Clearly such development required quite new sources and reception of information which we describe next.

The environment can signal to the cell *via* three main mechanisms since in the above description there are three very different informed systems, one based on the membrane, one on metabolism and one on DNA, which are dependent. First there is the system of all external metabolites, ions and non-metal compounds, connected to surface sensors and pumps, which can be affected by the environment. Second once inside the cell these chemicals, partly metabolised, can affect protein catalysts. The third is the linked back and

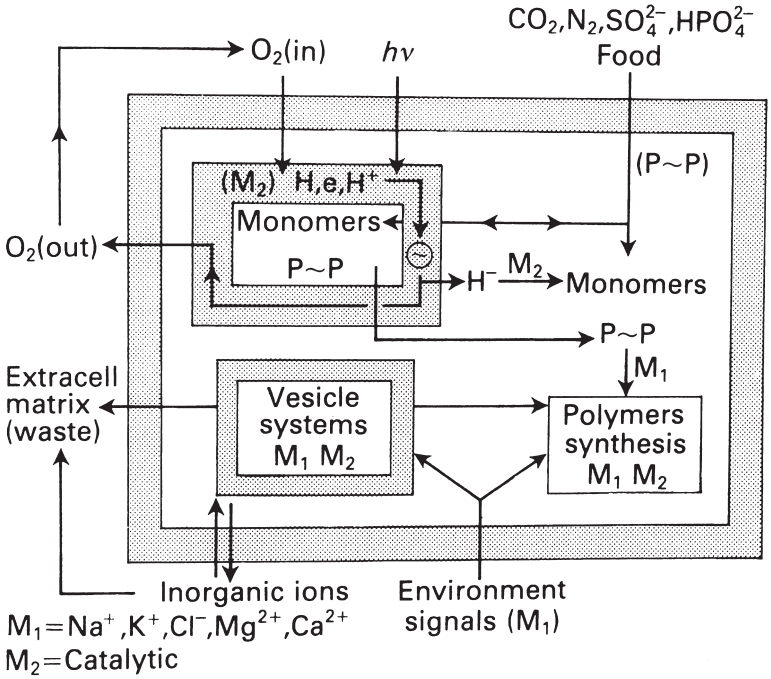


Figure 4. An outline diagram for the basic interactions in a eukaryote cell.

The response to the environment has increased.

forth response to metabolites or ions *via* proteins (transcription factors) which has DNA (RNA) as its information code and then proteins. The first and second type of signal can interact with the membrane and then go directly to the metabolic paths while the third goes to the transcription factors and the production of proteins. Of course the first and second signal can lead on to a

DNA (3), slow
↑
later third signal e.g. food → c-AMP
↓
metabolism (2), fast

Since the third requires protein production it is slow while the first and second are fast and require nothing more than a conformational change of pumps or enzymes adjusting rates of activity. Now the need for a fast response, such as change of shape or position, is obvious in an organism of long life, which wishes to capture others for food or to escape predators. It is especially necessary too for the rejection of stored poisons and proteins from vesicles for protection. It is also very advantageous if the same signals switch on energy quickly for these activities and switch on or off other required metabolic systems associated with digestion. There is no immediate need for novel protein synthesis opposite a new external situation if all these activities are capable of modulated rate through signals from the environment to metabolic rates. What is required is that the conveying material, the messenger, does not interfere directly with the pre-existing cytoplasmic operations and their signals and does not affect the DNA machinery. An ideal signalling solution was found in addition to those in bacteria.

When cells were first formed they had to be low in the calcium ion in the cytoplasm since it forms insoluble salts with organic anions. To avoid this circumstance Ca^{2+} ions in the cytoplasm were reduced to 10^{-5}–10^{-7} M while outside the Ca^{2+} ion was 10^{-3} M. This gradient can be used to stimulate inside the cell if (1) a sufficient input of Ca^{2+} can be generated by an event: the source of the signal is the environment. Note bacteria have very lit-

tle need to adjust behaviour as they have a short life before reproduction to which end their existence is dedicated. Their need is for nutrients only. (2) There are cytoplasmic receptors which link to activities; these receptors have to be selective for Ca^{2+} against Mg^{2+}, see references. (3) The signal can be linked to an internal condenser, a vesicle store of Ca^{2+}, Ca^{2+} release by Ca^{2+}. (4) There is a later rapid way of removing the ion that is a pump or sequestration so that it does not act as a poison. (5) There is a defined response or stimulation. The eukaryotes have a flexible membrane unlike the bacteria and an obvious stimulus is a deformation of that membrane on meeting an object. *This is a sense of touch*. We observe that eukaryotes developed this sense and that it gives rise to a calcium input pulse (signal) which triggers responses in a whole multitude of actions almost all those listed above, *via* receptor proteins such as calmodulin, Fig. 5, and various

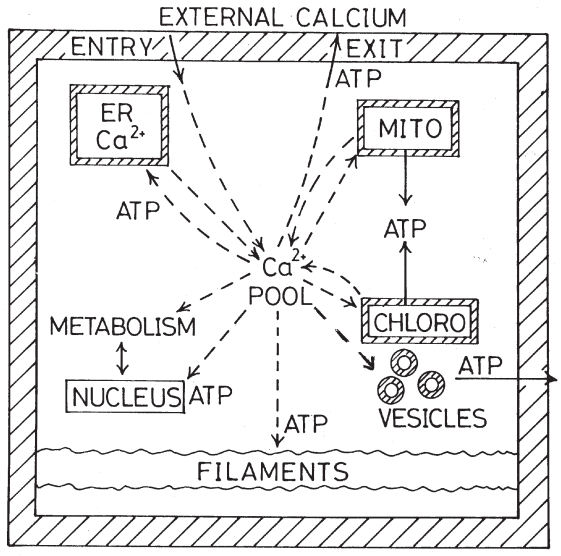


Figure 5. The communication network for pulsed Ca^{2+}.

In this case the on/off rates need to be taken into account as well as binding constants.

phosphates. It was helpful that touch discriminated between a desirable and an undesirable object by 'feel' and was linked in a different way to metabolism than smell. Remember, smell, a sensing of nutrients led to a c-AMP message in bacteria and this message now had an extra intermediate connection to calcium. At this stage of evolution light sensing (an eye spot) had also evolved in both plants and animals. It was used only to orient the organism and to direct it toward light intensity. The movement again involved a calcium release and in animals a message to the cell motors with a novel pathway using G-proteins. Calcium fluxes became increasingly associated with the interaction between single cells and their environment.

An important feature of the involvement of calcium is that it's ionic concentration in the cytoplasm had to be held low, Fig. 3, at a fixed value $< 10^{-7}$ M. This was managed by the feed-back signal from free internal calcium to the pumps which are switched on only when Ca^{2+} concentration exceeds 10^{-7}M. The pump then has a switch on control with a binding constant of 10^{7} M^{-1}. This is close to the value for binding to *all other calcium binding proteins in the cytoplasm* including here calmodulin and later troponin, annexin, S-100, and C-type sequences. The fixed constant for all the related activities compares with the fixed constants for Mg^{2+} ($10^{3.5}$ M^{-1}), Fe^{2+} (10^{7} M^{-1}) and later other metal ions Zn^{2+} (10^{11} M^{-1}) and Cu^{2+} (10^{15} M^{-1}). These and other values are characteristic of the mineral signalling and activities of the cytoplasm. They form the essential free metallome, Fig. 3, which is common to all cells. The selectivity of this binding, a feature of all cells, is discussed in the references.

Now as stated the creation of vesicles allowed selective storage of ions and chemicals and operation of new chemical pathways. The storage was of elements rejected to low concentration in the cytoplasm. This occurred without much genetic instruction since the vesicles are made in effect from invagination of the cytoplasmic membrane with resultant inversion of pumping. Thus vesicles are found in eukaryotes with stores of Ca^{2+}, Cl^{-}, Na^{+}, Zn^{2+}, Ni^{2+}, Co^{2+} but not with Fe^{2+} or

Mg^{2+}. Also organic chemicals could be stored there. Any such store is a source of a message but it requires energy to build it and a release mechanism. The release can be by calcium from outside releasing the material inside a vesicle to the cytoplasm or to the outside. (There are other release mechanisms based on phosphates or adenine derivatives liberated by the external event.) Thus initial information is amplified and interpreted later by this release. This type of amplification of the initial signal is found in early eukaryotes but developed greatly later.

The number of receptors for Ca^{2+} signalling both of one type and of different types is small, e.g. in yeast and already in yeast there are a small number of other new message systems using zinc and copper but as their signalling is much increased later we refer to it under the discussion of signalling in multicellular organisms. Zinc and copper were only available from an intermediate time of evolution. At this stage of evolution, e.g. in yeast, very little use is made of external/internal messages based on organic chemicals despite the introduction in slime molds of c-AMP communication between cells.

Table 2. Calcium in evolution

Cellular organization	Calcium function
A. Prokaryotes single cells	Externally – wall crosslinks – random mineralization Internally – very slow signal: swimming No message to genetic code except for sporulation under starvation
B. Early eukaryote single cells (No organelles)	As for prokaryotes (A) but external events relayed to internal structures (a) Shape response: contractile devices (b) Metabolic and energy response (c) Controlled external mineralization (e) Control over cell death

There is not much new signalling directly to the DNA (RNA) from the environment in unicellular eukaryotes though there are changes in the internal signalling in order to produce the vesicular and filamentous systems. Some of these changes are multiplied in multicellular organisms and we introduce them below. At this stage of evolution there are two obvious omissions, the possible use of the $Na^{+}/K^{+}/Cl^{-}$ external gradients and the use externally of organic molecules in signals. Virtually all other gradients of required elements $Mg^{2+}/Ca^{2+}/Fe^{2+}/Mn^{2+}/MoO_4^{2-}/$ and the small non-metal anions are used.

MULTI-CELLULAR ORGANISMS

So far we have described the signalling systems of a single cell eukaryote. Multi-cellular eukaryote organisms have quite additional problems. The whole organism starts from a fertilised special cell, this cell has to multiply differentially, and the organism must manage the environment. The requirement is for a completely new set of signals, which are not to be confused with the primitive but necessary messages in the cytoplasm of each cell and those from the more or less fixed environment of a cell now generated more and more by extracellular fluids. The signalling therefore had to coordinate separately the many slow processes of growth and the necessary fast responses of the whole organism opposite the environment. The multi-cellular organisms developed two new cell to cell signalling systems of *organic chemical signals* —

slow to and from the genetic apparatus with its coded DNA (RNA) for growth and fast to and from the metabolic pathways *via* the membrane or senses for environmental advantage, Fig. 6. As we shall see cells gradually became surrounded by a fixed internal extracellular fluid so that there was novel extracellular fluid/cell signalling. This signalling became dominated by a range of Ca^{2+} receptors, Table 2.

sity of the energy flux, though no sight. The transfer of information from light or heat to an activity depends on photoreceptors and temperature receptors which, as a result of many steps involving hormones, signal to the DNA of all cells through transcription factors. Energy from the increased light or heat then informs all protein synthesis throughout a plant. Here we see very clearly that protein receptors, with their origin at DNA, receive

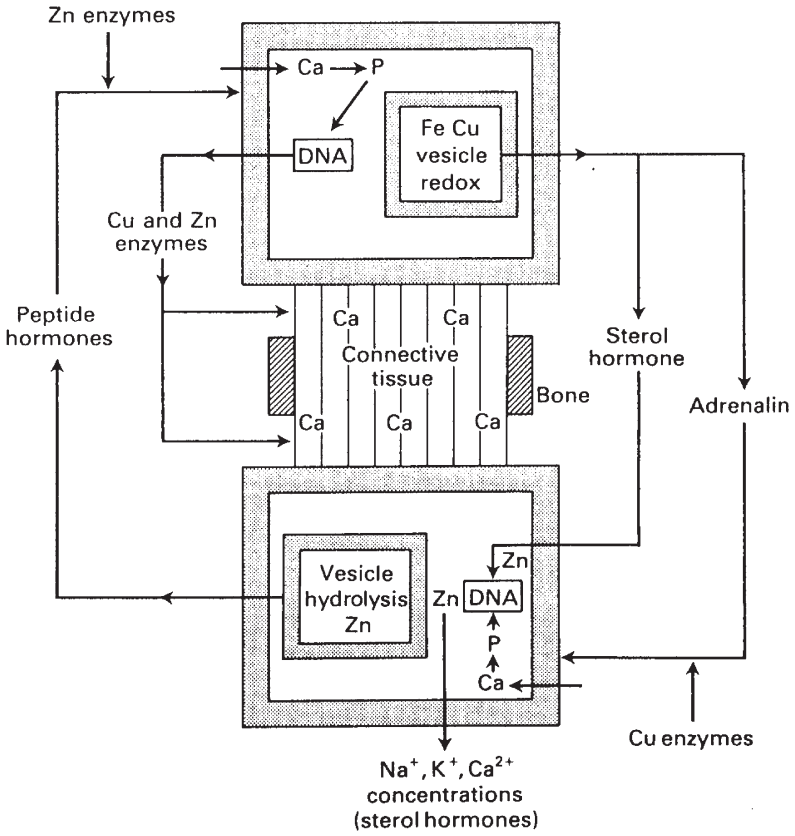


Figure 6. An outline of organic signalling between cells based on fast responses e.g. *via* **the extracellular fluids.**

It is well-known that quite early multi-cellular systems, before there was a nervous system, used organic molecular signals (hormones) and that this innovation was differently introduced in plants and animals. The main feature in plants, taking them first as they are easier to analyse, is slow control of growth and its timing. The mechanisms are often light and temperature dependent so that the organisms have sensors for the intentheir information from the environment in sensing cells and that these cells inform the DNA in many other cells *via* hormones to activate the whole plant. The feedback is also to the synthesis of new sensing and receiving devices for light so that light capture generates greater light capture. In the process the hormonal responses of plants couple to not just the persistent primitive internal messengers of the cytoplasm first seen in bacteria but the

external/internal signalling due to calcium seen in single cell eukaryotes, (hormones). In a plant the number of Ca^{2+}-receptors is still small but considerably greater than in single cell eukaryotes.

Fast: Organic Hormone A → membrane → Ca/P → metabolism

Slow: Organic Hormone B ⟶ transcription factors → DNA → proteins

Two signalling systems now act in parallel where P stands for phosphate compounds. One fast response is to energy generation coming from chloroplasts, which is sensitive to Ca^{2+} and Cl^- concentrations.

Let us now turn to a response of a hormone in an animal cell. Once again we need to see how signalling from one cell to another works. Lower animals, shell fish, do not have a very different response system to those of plants and it is interesting now to see how the organic chemical signalling especially in animals systems evolved from unicellular to multicellular organisms due to the availability of new elements, especially copper and zinc. This change came about as oxygen increased in the atmosphere, Fig. 7, in the following way.

Most of these organic messengers stored in vesicles. They are actually synthesised there using oxidative mechanisms and copper enzymes. Copper itself signals its presence through copper transcription factors to the DNA. The free copper level in the cytoplasm is close to 10^{-15} M, Fig. 3, and is too low for most communication purposes. It therefore has a carrier or distributing agent sometimes

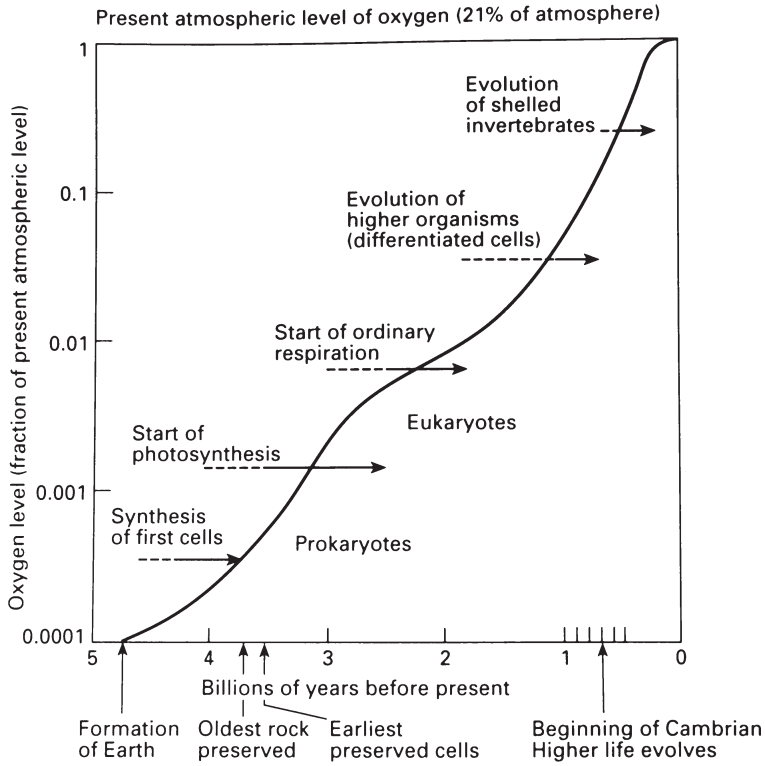


Figure 7. The correlation between the rise in oxygen in the environment and evolution.

Table 3. Organic messengers produced by oxidation

Messenger	Production	Reception	Destruction
NO	arginine oxidation (heme)	G-protein	? Heme oxidation
Sterols	Cholesterol oxidation (heme)	Zn-fingers	Heme oxidation
Amidated peptides	Cu oxides	(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? (*)	Se enzyme
Retinoic acid	Retinol (vitamin A) oxidation	Zn finger?(*)	?

(*) In the nuclear receptor super family of transcriptional receptors

wrongly called a chaperone. All the copper carriers, pumps and transcription factors have the same binding constants. Such carriers are known for calcium zinc and probably molybdate to aid distribution. In parallel with the use of copper in synthesis of hormones and of external cross-links was the development of zinc as an external agent for proteases designed to break connective tissue to allow growth and peptide hormones and for the messenger between growth transcription factors.

In the description of zinc internal signalling we turn to a different type of hormone from the fast acting hormones in both animals and plants and which is always present at low levels and to maintain growth but alteration of the signal affects growth. These hormones such as sterols, retinoic acid, thyroxine (ethylene?) are soluble in organic solvents such as

Table 4. Evolution of simple ionic equilibrium signals

Stage 1	Primitive organisms Prokaryotes	$Mg^{2+}/ATP^{4-}/HP^{2-}$ controls phosphorylations Fe^{2+} controls redox equilibrium $Na^{+}/K^{+}/Cl^{-}$ control osmotic pressure
Stage 2	Single-cell eukaryotes	Ca^{2+} controls activated states and relationship to environment Mn^{2+} controls development of plant-related organisms
Stage 3	Multicellular	Zn^{2+} controls hormonal responses relating to growth of organisms and development and connective tissue. $Cu^{+}(Cu^{2+})$ controls connective tissue and responses Extended use of Ca^{2+} in excited states Generation of Na^{+} (K^{+}) signalling and evolution of the nervous system

control of growth hormones at zinc fingers, Table 3. Zinc is held at approximately 10^{-11} M for binding to its pumps, carriers and transcription factors and as a general membranes and pass through them to the DNA/RNA. They interact directly with transcription factors of a new kind – zinc fingers. The activity induced is extremely slowly

changed during growth so that there are various metamorphic events in an organism's life. The change is due to growth itself – growth signalling to how to grow. These new hormones must act in harmony. We have seen how the internal mechanisms of primitive cells generally are controlled by ions such as Mg^{2+} and Fe^{2+}, the concentrations of which are quite fast signalling messengers. The zinc fingers cooperate through the slow exchange of zinc so that this cation becomes a new messenger inside cells and even outside cells, Table 4.

THE SENSES

A big step forward in evolution of signalling was in the development of sensing. The sense of smell, touch and light intensity arose in succession *via* prokaryotes and single cell eukaryotes as we have explained. The big advance in multicellular organisms is sight – an ability to place objects in space as well as responding to light intensity. Closely in time there developed hearing, an ability to place objects in space due to sounds. Both these senses require a coordination not just of incoming information (signals) with a muscle response but an ability to build an internal three dimensional map corresponding to the external data. In other words they have to be able to collect data and integrate it in the brain. Clearly this requires an advance of signalling on the octopus or nematode nerve responses. The detailed nature of the environment is signalled to the brain *via* energy transfer initially. The organ for collecting and integrating signals is the brain.

Now the brain also has the ability to remember an image and give it attributes distinguishing the type of object, which is seen or heard as well as smelled. How is this achieved? We are asking how do nerve cells accumulate signals. They do so by the stimulation of growth of cells to contact one another on receipt of a message. In this way an external image is converted into a topological map of fixed gradients of charge and chemicals. The environment is now represented by coded information not in DNA. Activation of the gradients at any time recreates the image within the brain even in dreams. It also allows recognition of all aspects of the environment. The network of gradients is in storage of charge (ions) and of organic chemicals relative to the environment – here the extracellular body fluids.

THE BRAIN SIGNALS

The scavenging activity of larger animals needed more rapid internal long range response to coordinate senses and muscles at least as much as slow response using DNA and protein synthesis. To meet this demand animals, such as jellyfish, evolved elongated nerve cells to add to the message systems. These nerve cells use the $Na^+/K^+/Cl^-$ and to some extent Ca^{2+} ion gradients to conduct current, a new message system, over long distance so as to connect organs remote from one another. Note that all the gradients used in this electrolytic signalling are present in the earliest cells and are related to the steady state metallome of all cells, Fig. 3, but that the small size of primitive (bacterial) cells and single cell eukaryotes made such long range electrolytic transmission of no value.

To describe the essence of nerve signalling in more detail we have to make a further comment about nerves. Nerves depend upon Na^+/K^+ gradients for conduction – they convey a message but not activity, as they have no direct receiver. In fact the binding strengths of their single ions and Cl^- are so weak generally that by themselves they can control only small protein changes such as the opening of channels by cooperative action as a potential. The signal is a cooperative action of a potential change. At a nerve terminal calcium channels are therefore opened by this electrolytic potential message and Ca^{2+} en-

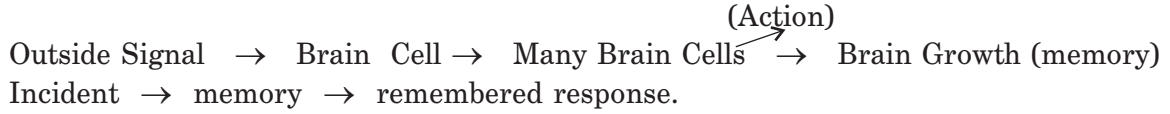
ters the cell. Then the nerve terminal vesicles, activated by Ca^{2+} influx, discharge organic transmitters or hormones. The eventual action is relayed for example with feedback from muscle (even by self-observation). This is the most primitive signalling of brains

Environment → Nerve → Brain → Nerve → Muscle

Cell I Cell II

Now in advanced animals the brain is not just a relay since as a consequence of the messages the nerve cells of the brain grow creating a three-dimensional coded memory – information store or source. Once again we see the feedback nature of signalling

next step involved cell–cell signalling in multicellular organisms for time growth and later activity. Senses allow much new information from the environment. A diversity of new signalling systems evolved to relay information from cell to cell and from the outside to the organism. These signals also generated active responses. Note that on reaching the membrane of any cell signalling is dominated by calcium and then by the primitive internal signals for any fast response but for growth a quite new signalling system arose dependent on zinc ions as well as a different group of organic signals, hormones. At each stage we observe the number of calcium and zinc systems increasing rapidly. The final evolution step was that of the brain with its ability to handle vast amounts of environmental data and act accordingly. The novelty in signalling was now the Na^+, K^+, Cl^-, (Ca^{2+}) potential which needed a relay of calcium and organic signals to cause change.



Outside Signal → Brain Cell → Many Brain Cells (Action) → Brain Growth (memory)
Incident → memory → remembered response.

In the end the action is stopped but not without leaving an almost permanent imprint. This development allows the arrival of *the phenotype* with now a great deal of information independent from the gene, *the genotype.*

SUMMARY OF THE EVOLUTION OF BIOLOGICAL SIGNALLING

We need to see that the evolution of biological signalling initially largely inside cells to organise the cell was followed in stages. The second stage was two fold. Cells evolved which were larger in size, had flexible membranes and internal compartments with the environment, Fig. 4. The new signalling, which reflected the state of the membrane, that is information about the external environment, and the relative states of the internal compartments, had to be different in kind from the primitive internal signalling. The calcium ion gradients fulfilled the need as messengers. The

The above signals concern the metabolites and the environment although growth of cells also involves the DNA. Now higher organisms also have new use for slow processes, Table 3. Finally we draw attention again to the remarkable involvement of calcium in an every increasing way in eukaryote signalling, Fig. 8.

MANKIND AND SIGNALLING

Mankind has taken signalling and control one step further in that it has advanced to or-

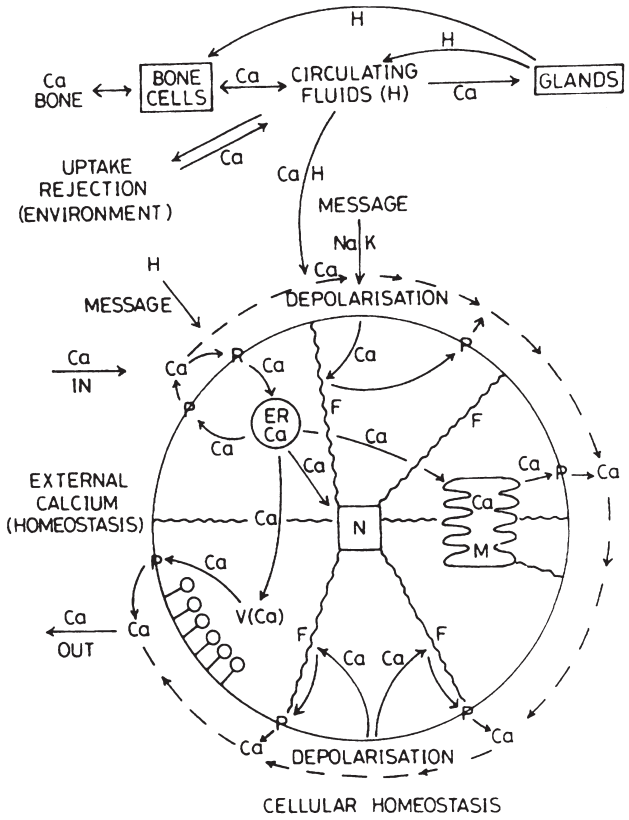


Figure 8. The essence of calcium signalling in a higher animal coupled to the fast responses shown in Fig. 6.

ganism/organism signalling on a grand scale. Initially communication between two animals was probably based on sign language which became various forms of coded signal e.g. flag-signals. Later sound was developed into language. Language was coded into electronic and energy (light, microwave) signals making use of new materials and energy as sources. The signalling based on computer language would appear to be similar to that of the brain. Now a computer uses the all or none signals 0, 1 in sequences such as 0, 1, 1, 0, 0 and the receiver interprets such coded material in an all or none way. Note now the quality difference from the brain signals in that *cellular information is transmitted by the intensity of binding relative to the signal molecule concentration.* Each unit has an intensity unlike 0 or 1 which are all or nothing. The receiver grows to affect the behaviour of the organism gradually. Thus the image for a human brain is adjustable by experience in an uncertain (individual) way. It is also connected by inheritance to previous experience of organisms back in time since the internal responses of the cell are much as they always were. It is not a strictly reproducible response to an event and the level of predictability of the response becomes of great uncertainty. It is individual in mankind – in part inherited, genotypic, and in part from experience, phenotypic. Obviously mankind has developed signalling to an extraordinary level and is bringing about cooperation in large societies. The advance of signalling is the advance of the phenotype relative to the genotype.

SUMMARY

Signalling is not easily appreciated. It is not a simple matter of the fixed content of a mes-

sage but is dependent upon the intention of the donor and the response of the receiver before there is information. At first cells received few messages except by internal exchange. Later as eukaryotes they received more information from outside while developing internal systems. The outside became more significant as cells became able to signal to one another and to gain knowledge of the environment. However, no matter what changed there is a residue of the earliest history of cellular development in all present day species. Evolution is clearly of systems organisation which demands greater and greater dependence on signalling to gain cell cooperation no matter how large the number of cells or even organisms. Peculiarly in animals the brain evolved a mode of signal reception which gave internal pictures of the environment. In turn this allowed the brain to correlate and compare and finally to construct externally. Some responses to the environment now used no reference to DNA machinery since the brain can act as a directive organ using its own store of information accumulated from signals.

REFERENCES

The literature on signalling is immense. The novelty in this paper is derived from consideration of systems. We direct the reader's attention to our own work only as a starting point, which can be followed in these references.

Fraústo da Silva JJR, Williams RJP. (2001) *The Biological Chemistry of the Elements,* 2nd edn. Oxford University Press, Oxford.

Williams RJP, Fraústo da Silva JJR. (1999) *Bringing Chemistry to Life.* Oxford University Press, Oxford.

Williams RJP, Fraústo da Silva JJR. (2003) Evolution was chemically constrained. *J Theoret Biol.*; **220**: 323–43.

Full coverage of various aspects of signalling is to be found in the handbook:

Bradshaw RA, Dennis EA. (eds) (2003) *Handbook of Cell Signalling;* Vols 1–3. Elsevier, Amsterdam.