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# Calcium: outside/inside homeostasis and signalling

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**Abstract**

More and more data accumulate concerning calcium dependent effects in all compartments of cells. The higher the organism in evolution the more calcium becomes involved. Inspection of the data while looking for an explanation of the involvement of calcium in metabolism, nuclear functions, control over pumps, external activities, mineralisation and so on leads one to suppose that calcium has an integrating function. The implication is that calcium flow is a large network connecting the environment, the cytoplasm, vesicles, organelles, the nucleus and in higher species, organs. There is the possibility then that calcium ion functions are being analysed, often in vitro, in a bit by bit reductionist manner while in vivo calcium is the equivalent of an electron in complicated electrical circuits. We then should look for its connections to energy, to effects where conformational switching by calcium pulses is equivalent to magnetic triggering by electron flow and where storage in vesicles is equivalent to condenser-like devices and so on. The appearance of pulsing, of time delays in parts of circuits, and other properties of electronic circuits seen in calcium triggering are then explicable as part of calcium circuit design. No other ion can operate in the same way due to the peculiarities of the calcium ion, its size, charge, ionisation potential and its availability which allow it both to flow rapidly yet to bind considerably. 

*Keywords:* Homeostasis; Calcium circuit; Compartment; Bio-energetics; Environment; Cell control

## 1. Introduction

In my last lecture at the International Meeting on Calcium Proteins, in Lund in 1997, [1] I stressed features of calcium activity which were linked to the activity of a range of other elements including $Na^+$, $K^+$, $Mg^{2+}$, $Mn^{2+}$, $Fe^{2+}$ and $Zn^{2+}$ among metal ions and phosphate especially amongst non-metals. I stressed that these connections are part of a general link in all eukaryotes between the activities of the cytoplasm of a cell, of the external environment and of the content of organelles and vesicles, which were all related to calcium levels. The further suggestion was that much of homeostasis of a multi-cellular organism is also monitored by calcium controls. In this respect calcium is the dominant messenger for triggering, which crosses membranes of all kinds, while it is also a homeostatic control for both the intracellular and extracellular fluids of higher multi-cellular organisms. Its activity differs greatly from the more primitive messenger uses of hydrogen ions, substrates, phosphate and iron compounds which already in prokaryotes act as the major homeostatic controls and triggers of the cytoplasm alone. However, the innovation of the use of calcium in external and vesicle communications with the cy-

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toplasm of eukaryotes had to be married to the use of these internal earlier messenger systems of primitive cells since much of eukaryote metabolism is carried over from prokaryotes. We therefore found a cross communication network of messenger systems which employ calcium, hydrogen, substrates, phosphates and iron compounds in the cytoplasm and in the interior of organelles of eukaryote cells. In this article I shall continue this theme of calcium functions connected to those of many other elements but at the same time I shall look at energy use and distribution often involving ATP and where calcium is involved. I shall be stressing that from soon after the time of the appearance of eukaryotes the role of calcium was in the management of cell homeostasis through *continuous energy production* and distribution as well as that of material transfer and metabolism, while acting as a trigger messenger across all membranes when required. This flow of energy is an essential part of homeostasis. There is then an evolutionary progression in the use of calcium in a series of add-on steps:

1. External use in outer cell walls and membranes of the most primitive prokaryotes
2. Messenger for protection and external responses of single cells of early eukaryotes generating contraction and similar responses, through binding to internal matrix fibres, e.g. actinomyosin
3. Internal energy homeostasis in single cells of early eukaryotes linking organelles, vesicles and the cytoplasm
4. External structural and messenger use for interactions between cell connectivity of multi-cellular organisms

I dismiss (1) the use of calcium in primitive prokaryotes, at least where it acts merely as a protective external cross linking agent, as of little in consequence in this article. Undoubtedly today some prokaryotes can use calcium gradients in message systems too but the constraint in a prokaryote is that it must multiply rapidly rather than constantly adjusting itself to environmental stimuli so that even here the use of calcium is not very extensive. Growth is merely a doubling in size before division. It may also be that calcium was used early in sporulation again in a protective salt. Prokaryote internal metabolism is relatively flexible much though it is connected to hydrogen, substrate, phosphate and iron controls. This can be seen by looking at those cytoplasmic metabolic paths common to all organisms and including some, as in the following example, which remain largely independent of calcium to this day even in advanced eukaryotes.

Energy directly from sugars without oxidation is gained in the well-known glycolysis pathways by prokaryotes and eukaryotes alike. Here the products of glucose internal disproportionation are pyruvate and then acetate: $NAD+(CHOH)_n \rightarrow CH_3.CO.CO_2H$ $(C_3H_6O_3)+NADH \rightarrow CH_3CO_2H$ $(C_2H_4O_2)$ acetate. The reaction is a special case of energy production and of the general control of internal metabolic pathways of all cells by catalysts not linked to external or internal conditions by calcium messages. In *prokaryotes* the control by feedback not only of this pathway, but its links to fat production and degradation, to synthesis of amino acids and nucleotide bases and its connection to the citric acid cycle are all managed by the levels of hydrogen, substrate, phosphate and iron compounds without calcium. We need first the background to this control activity linked to energy before we consider the role of calcium in eukaryotes.

## 2. Homeostasis

A cell is characterised by activities encoded by DNA which is read by a machinery and then transcribed and translated. The activity, including dynamic structures, is seen in properties of a large number of large and small molecules which can be examined separately in vitro but in vivo their properties are all related (Fig. 1), so that the activity forms *a pattern of flow* of material and energy. The fixed pattern in steady state, which is present both in physical form (of all materials) and energy flows, implies that in the steady state all chemicals in the cell are in fixed ratios. The maintenance of this composition is called homeostasis and since the cell is always active in many ways it needs many controls but at the same time all the activities have to be integrated. Thus its possible variables need to be controlled by coordinating messengers of all activities of the cell.

Now we need to define more clearly what are the variables which have to be controlled. Chemicals are

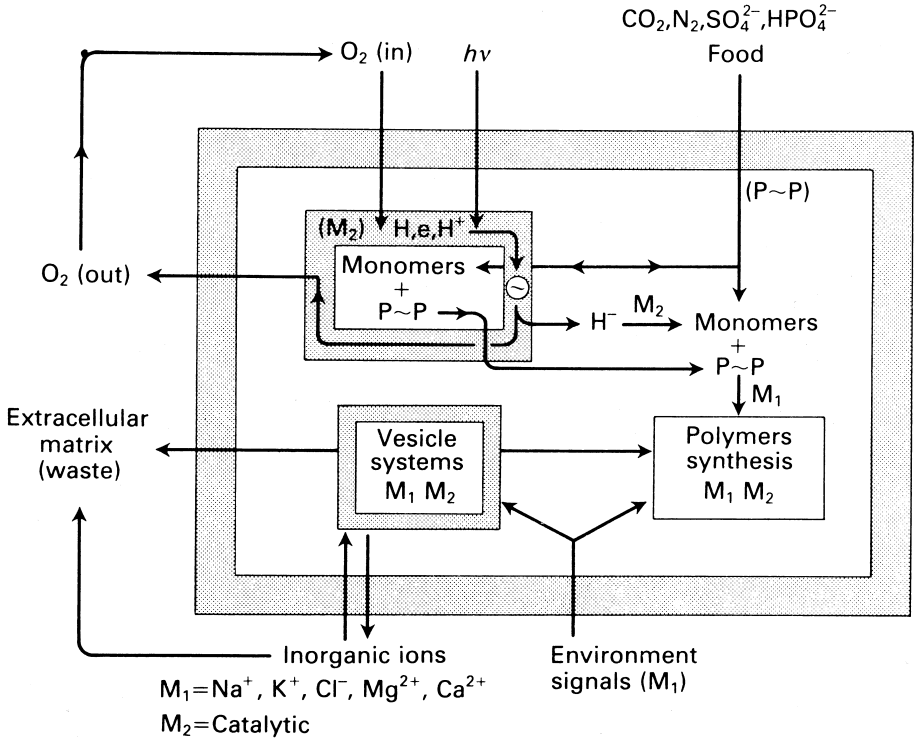


Fig. 1. The generalised pattern of activities, flow of energy and material in a cell.

all just combinations of atoms but their combinations contain different amounts of energy when referred to element standard states. For example, $CH_3COOH$, $CH_4+CO_2$ and $2C+O_2+2H_2$ all have the same element composition but differ in energy content ($\Delta G$) (see Fig. 2). If all three chemicals were in equilibrium then the ratios of each of the three compositions would be fixed at any given temperature and pressure. The variables are then the concentrations of the elements, C, H, and O, and energy incorporated and not those of the compounds $CH_3COOH$, $CO_2$ and $CH_4$ or any others of the same composition, per se. Now the cytoplasm of biological cells is not in equilibrium but in fixed homeostatic flow. In fixed flow the ratios of say the above three combinations of C, H and O are maintained both by controls on the amount of the elements and by the energy *entering and leaving the cell* both of which leave in different form from that in which they enter. The flow has restrictions, catalysts, which decide the compounds formed in the cell, and cells must also have controlled osmotic and electrical solutions. These catalysts and the composition of the aqueous milieu require many elements other than H, C, N and O. The variables, not directly the amounts of individual combinations of elements such as $CO_2$, $CH_4$ and $CH_3COOH$, in a cell, must therefore be constrained by the control feedback over uptake (and rejection) of many elements and intake and loss of energy plus controls over their distribution. Thus the entry and exit of metabolic, catalytic and control chemical elements, some twenty, are the variables of composition and the actual composition of chemical compounds in the cell is dependent upon the energy another variable which is put into these elements in combination. Consider first the homeostasis in a prokaryote.

The way in which prokaryote homeostasis is managed is to have (1) a set of catalysts which see to it that the reactions of a given substrate, e.g. glucose, connect only to a special pathway and (2) a set of ions to maintain fixed solution conditions and rate constants. The pathway rates, for example glycolysis, are such that the path is decided both in direction as is a walking path, and in rate by a series of selective reduced energy barriers to chemical change, compare styles in hedge-rows on walking paths, by catalysts. Now to make sure the flow is maintained at a steady

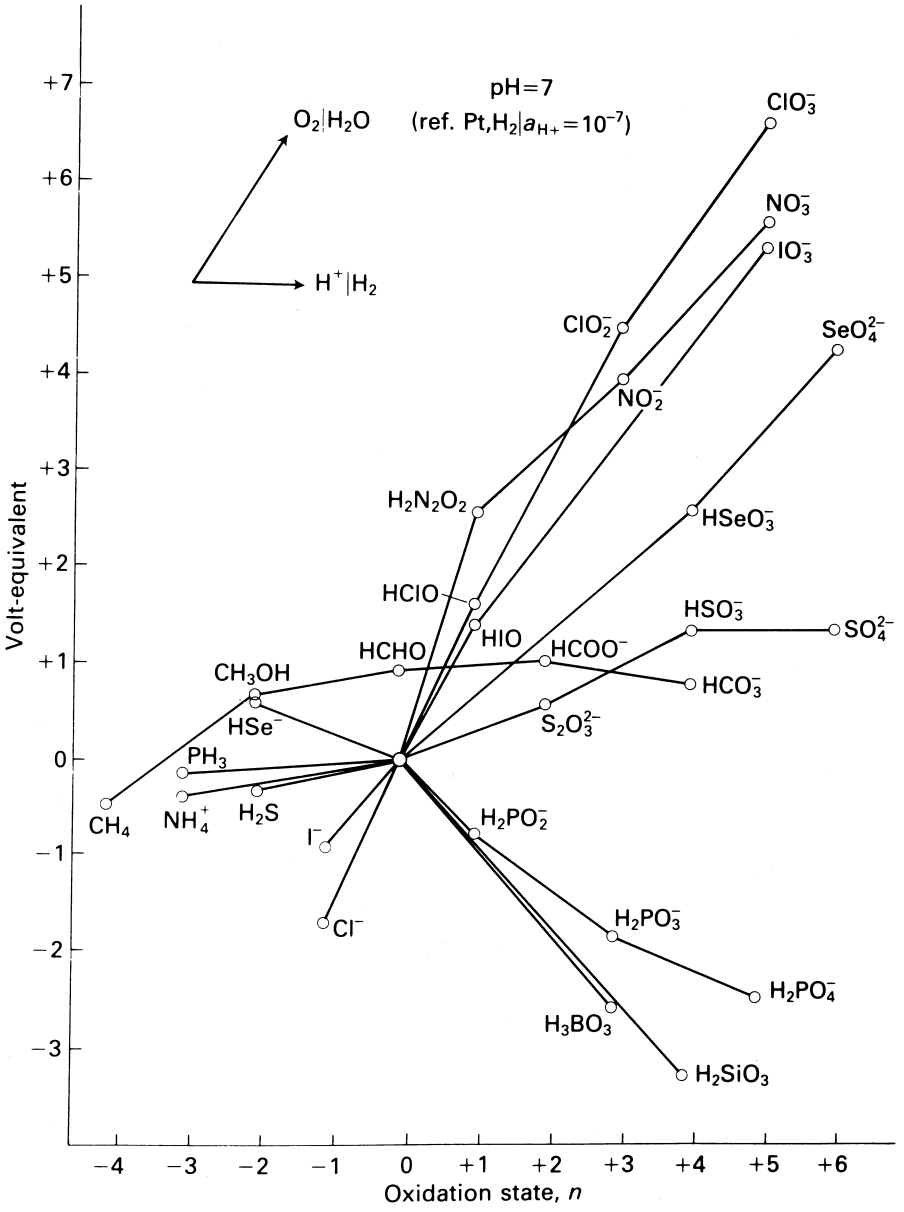


Fig. 2. An oxidation state diagram for carbon showing that all carbon compounds other than $CH_4$ in the presence of $H_2$ and $CO_2$ in the presence of $O_2$ are unstable. Cellular life turns over quite unstable carbon compounds constantly and requires constant input of energy and material. These flows need constant controls. Oxidation state diagrams are given for other non-methals for comparison.

rate the products of later steps are fed back to adjust the catalysts, reducing or increasing their efficiency. Glucose flow to pyruvate is then a steady-state flow managed by products including pyruvate. Now the products of this pathway connect to other pathways such as amino acid synthesis, fatty acid synthesis and degradation and so on. Each pathway has its own internal feedback (and feedforward) controls and hence a fixed rate. The cell must also have continuously operating uptake and rejection mechanisms. However, to hold the whole cell in homeostasis the different pathways must be controlled in rate relative to one another. This is managed in the cell cytoplasm by using co-factors, common to different pathways, as controls over enzymes in more than one pathway – examples are NADH and ATP which distribute hydrogen and energy (common needs of many pathways) but also act as common feedback (feedfor-

Table 1
Connections to ATP or gradients in anaerobic prokaryotes

| Reaction | Connection |
|---|---|
| Glycolysis | ATP production and feedback restriction |
| Fe/S systems | Production of $H^+$ gradients prior to ATP formation |
| Ion pumps | Rejection of $Na^+$, $Ca^{2+}$, $Cl^-$<br>Inward movement of $K^+$, $HPO_4^{2-}$, $Fe^{2+}$, $Mg^{2+}$, etc. |
| Exchange for $H^+$ or $Na^+$ | Inward movement of substrates |

ward) allosteric controls over enzymes in many pathways. There are other coenzymes for distributing carbon and nitrogen. This means that a steady state can be characterised by a level of such coenzymes as NADH and ATP but also all the intermediates in all paths so the resultant cell is fixed. The cofactors have to act on the catalysts. However, while some pathways generate energy others require it. Now several *co-enzymes connect to energy* – either $H^+$ gradients or ATP itself – and then are related to pathways. To keep all the elements in balance input and exit pumps and exchanges of all required elements for catalysts and control of ionic solution conditions (Table 1) also must be connected to ATP or $H^+$ flow. The result is a steady flow of chemicals through membranes and the cytoplasm while small amounts of material and energy are syphoned off to make DNA keeping the protein complement roughly constant in composition. The overall result for a prokaryote is just rapid reproduction against a background of almost fixed metabolic activity, i.e. of homeostasis – a totally dynamic (flow) concept involving 20 elements in cyclic flow and an almost fixed flow of energy degradation (Fig. 3).

The managed required elements as well as H, C, N and O of almost all if not all prokaryote cells are S, P, Cl, Na, K, Mg, Mn, Fe, Co, (Ni), (Cu), Zn, Mo (W) [2]. All are held at closely fixed concentration ratios within any cell. Since there are always losses input must be constantly maintained and monitored. This state of affairs can only be procured by the fixed *division of the use of energy* seen by the level of ATP. We see that *the essence of total homeostasis is that certain substances, mainly coenzymes and simple ions, form flow (currents) connecting the pathways in circuits* (Fig. 4). The currents are not shown in the pathways but we give biochemical charts much as is usually the case in electronic circuit diagrams. There are, however, flows with different rate constants in different parts of the circuit, e.g. of phosphorylation and in the feedbacks between large molecules (see Table 3) [3,4].

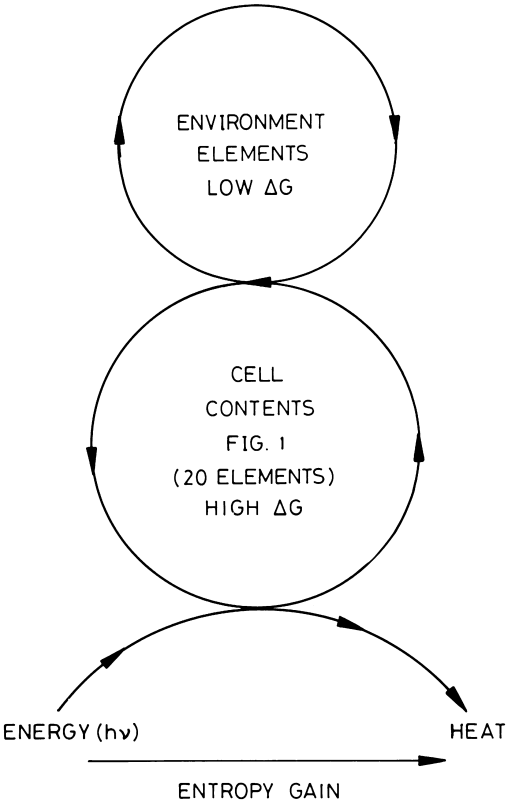


Fig. 3. A scheme for the maintenance of cellular flow. Overall a cell cycle involves energy build up with an average steady state of $\Delta G$. This $\Delta G$ must be constantly renewed from the environment as compounds degrade. To maintain the condition energy degradation, creating entropy, is needed e.g. from the sun to yield eventually heat. Hence the application of energy as well as basic material to synthesis needs constant control.

Table 2
Calcium in organelles+Golgi

| Organelle | Activity of calcium |
|---|---|
| Chloroplast | Associated with dioxygen release (Mn, Cl) (Fe/S) |
| Mitochondria | Associated with dehydrogenases (Fe/S) (Mn?) |
| Golgi | Associated with folding (Cu, Fe, (Zn)) (Mn and glycosylation) |

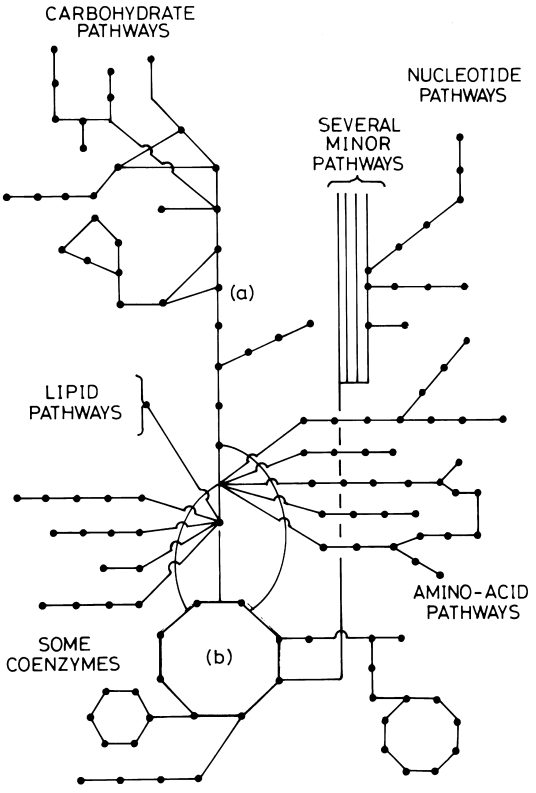


Fig. 4. A deliberate attempt to liken the metabolic pathways of a cell to the outputs of a computer circuit: (a) glycolytic pathway, (b) citric acid cycle. All such circuitry which is here of basic H, C, N, O metabolism must have controls (see text). (After Kauffman.)

In the prokaryotes the energy distribution, which we have linked obviously and directly to ATP, does not involve calcium except that the ATP is used to pump this element out down to a level of about $10^{-5}$ M minimally, while pumping in and out various other ions and molecules [1]. As stated before we believe that the role of calcium in early prokaryotes was relatively trivial. (Note that prokaryote flow has a certain flexibility due to the ease with which new bits of circuitry can be made (synthesised) and inserted, e.g. when the external food supply is switched say to lactose.)

Now in the above we have referred to the control of the major hydrolytic and synthetic metabolism using H, C, N and O substrates and linked to chemical energy (ATP) and ion gradients. There are further reaction pathways in the earliest known anaerobic prokaryotes which utilise redox reactions. Their control involves message systems especially utilising sulphur and iron compounds in signalling so that redox homeostasis is managed. Probably all other essential element uptake and management are linked to a combination of these major controls. However, once again there does not appear to be a significant involvement of calcium but we have to observe that due to leakiness of membranes even at this stage of cellular life there is a constant calcium current to and fro across the cytoplasmic membrane. It is the evolution of this and other calcium circuits and currents which we shall follow in this article.

## 3. The development of eukaryotes

We start discussion of the eukaryotes from the assumption that they developed from anaerobic prokaryotes. The great advantage of the eukaryote is the division of cellular space into compartments which separate activities and which are held together by filaments in tension. The filaments and their possible changes in tension allow a great increase in size and an ability to change shape of a cell and make possible flexible external membranes used even to consume prokaryotes. All these additional activities require energy and it therefore has to be distributed and controlled sometimes in bursts of activity while it is constantly used at rest. During their longer lifetime the eukaryotes must then have careful internal

Table 3
Timed responses

| Time | Coupling | Response |
|---|---|---|
| $10^{-3}$ s | ATPases | Contraction (tension) |
| $10^{-2}$ s | Kinases and phosphases | Metabolic pathways |
| seconds or longer | Transcription factors after phosphorylation:dephosphorylation | DNA production of mRNA and proteins[a] |

[a]The activity of $Ca^{2+}$ in the nucleus is closely linked to phosphate mobilisation. It may well be that nuclear systems like those of mitochondria and chloroplasts are based on differently timed response [3,4].

control of vesicles, filaments and cytoplasm while they also suffer and respond to environmental changes. The great disadvantage of eukaryotes is that they live longer and, since they develop, they must protect themselves before reproduction. At first, they used the flexible adjustment just to assist actions such as endo- and exocytosis to eat and to attack enemies. They had therefore to be aware of their environment and to obtain signals from it. We observe that the *anaerobic* eukaryotes developed calcium flow in new circuits simultaneously with the novel introduction in them of useful filaments and vesicles, to aid maintenance and changes of shape. Calcium was chosen as the messenger because it had little previous use and because of its intrinsic properties [2] but also because it had already been rejected from the cytoplasm of prokaryotes to give a considerable gradient. In other words calcium managed the new homeostasis of internal cell tension and the new triggering of cell shape and both endo- and exocytosis using vesicles. Of course this required refinements in the handling of calcium inward and outward movements, channels and pumps, which had to be placed in the plasma membrane. Later we can assume that amplification of action came about using concentration of calcium ions in the vesicular systems such as the endoplasmic reticulum needing further pumps and channels and storage proteins. As this reticulum developed it could protect the new nucleus and signal to it also.

In our previous article ([1] and see above), we showed that this use of calcium as a messenger in the cytoplasm connecting the outside environment with the cytoplasm, the vesicles, the filaments and the nucleus needed also to link to the pre-existing (prokaryotic) anaerobic cytoplasmic controls. The cytoplasm is controlled as stated above mainly by substrates, phosphate and sulphur compounds, iron and hydrogen ions, which were inter-linked. Calcium had then to be linked to all of these control signals. We know that today it interacts directly with the signalling phosphate system, the energy system, and the ATPase pumps even for calcium itself. Phosphates interact with all the other primitive controls. The signalling network is then complete for the basic *anaerobic eukaryote steady state by a calcium circuit but calcium could also be used to trigger.* The calcium current flow noted in prokaryotes has been made much more sophisticated even in the resting state since it has localised features due to the disposition of pumps, channels and storage devices which fluctuate with the shape of the cell. The control over shape and then morphogenesis by local calcium currents is beautifully illustrated in work on acetabularia [5,6]. This situation was made more complicated by the introduction of the two more effective ways of obtaining energy by *aerobic* eukaryotes: the incorporation of *prokaryotes as organelles* in the form of chloroplasts and mitochondria. Now we have stated that these prokaryotes themselves did little with calcium! How did calcium come to link incorporated prokaryotes in the network of controls of eukaryotes?

Before we progress further we note that whereas early signalling in the cytoplasm is based on relative concentration of products of reaction, i.e. such ratios as [ATP]/[ADP][P], [NADH]/[NAD][$H^+$], and $Fe^{2+}$/$Fe^{3+}$, the calcium signal is based on the gradient ratio $[Ca^{2+}]_{out}/[Ca^{2+}]_{in}$. We assume that in evolution the calcium gradient increased (see [1]) by lowering in-cell calcium to $10^{-7}$–$10^{-8}$ M in eukaryotes from some $10^{-5}$M in primitive prokaryotes so enabling effective signalling. This use of a strong gradient does not appear to have developed for other available ions $H^+$, $Mg^{2+}$, $K^+$ or $Cl^-$, much though smaller gradients are used in later eukaryotes in nerves etc. Calcium in life is unique for good chemical reasons. It is the only *available ion* which gives *fast, relatively strong, reversible binding* to organic materials and fast diffusion. We note that if this summary is correct the incorporated organelles always could manage a higher calcium content than the eukaryote cytoplasm.

## 4. Homeostasis and energy of aerobic unicellular eukaryotes

As stated homeostasis in eukaryotes is more complicated than in prokaryotes since the new internal compartments, vesicles or organelles, all have to be in communication with the cytoplasm. Now homeostasis is a dynamic condition and costs energy. In order to maintain element homeostasis in eukaryotes much energy is required therefore in pumping ions, energy itself, and material across many membranes.

For a steady state this means that cytoplasmic ATP levels must be kept approximately constant so that all gradients across membranes are fixed. ATP is largely maintained in aerobic eukaryotes by new processes: the fundamental production of proton gradients in both the mitochondria and chloroplasts giving the cell ATP. Homeostasis (and triggering) demands signalling to and from these organelles in both resting and triggered states.

The activity of chloroplasts or mitochondria depends on the supply of light and $CO_2$, or dioxygen and reduced carbon metabolites respectively. Let us combine and describe both organelles as having a fundamental exterior energy supply, hν and $O_2$/hydride respectively, which is converted to a proton gradient *or* used to generate reduced carbon (plants) or heat (animals). We should like to know the way in which light or dioxygen use can be controlled. One general control apart from the activity of phosphate compounds appears to rest with the calcium levels in mitochondria or chloroplasts.

Now calcium is high when a eukaryote cytoplasm has been activated by a calcium input from outside the cell or from the reticula. The activity induced invariably uses ATP. Hence it makes good sense to gear up ATP synthesis while calcium is pumped into mitochondria as a partial recovery from activation. In mitochondria calcium moderates the activity of the dehydrogenases which produce ATP. We may therefore presume that homeostasis of the mitochondrial activity in relationship to the cytoplasm is restored and maintained by calcium input followed by mitochondrial $Na^+/Ca^{2+}$ exchange or by ATPase pumps for calcium. (We also know that excess calcium can so damage mitochondria that the release from them of cytochrome *c* causes apoptosis.) Note that the calcium ion relationship to the mitochondria is very localised [7].

The chloroplast supply of energy from light is also modulated by calcium which interacts with and increases the dioxygen production of photosystem II. This is due to the level of calcium inside the thylakoid. It may well be that the lower the use of light to give dioxygen, the greater percentage use to give reduced carbon by photosystem I. If the electrons are transferred to photosystem II rather than giving reduced carbon then more ATP is produced rather than stored hydrogen. A balance is needed between the two. High ATP levels outside the thylakoid will help to restore the state of the general homeostasis. Does calcium modulate this redistribution constantly hence keeping homeostasis between chloroplast and cytoplast in the resting state?

The suggestion then is that across the mitochondrial or chloroplast membrane there is always a gradient of calcium. The calcium is higher in the organelle – after all it is a prokaryote and can tolerate higher calcium. Modulation of this gradient sensitises the supply of ATP due to dioxygen or light supply respectively. According to need, say the demands for ATP, the calcium level in the organelle rises switching on ATP production. We are not suggesting that calcium ions are the only controls since we can be sure that for example free manganese and chloride levels are also important in both organelles (Table 2). In this article, however, the stress is on the network of calcium flows which now includes the reactions of the organelles and the ATP production for the cytoplasm (Fig. 5). The flows, currents, of calcium and

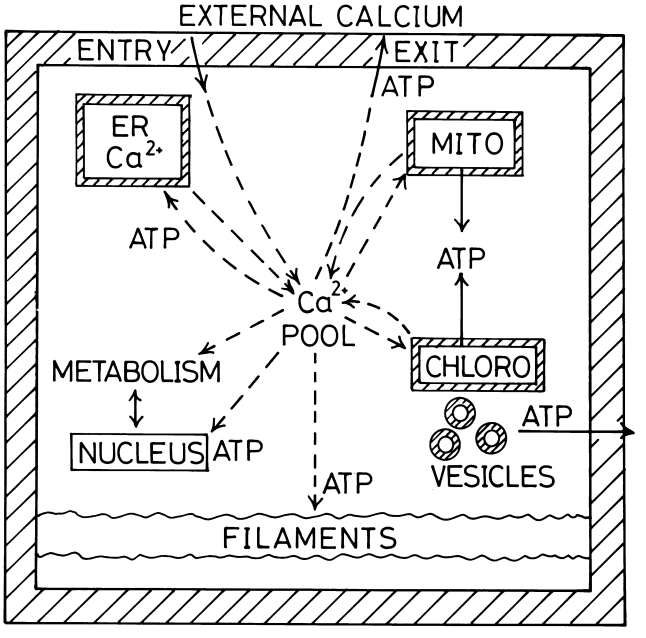


Fig. 5. The involvement of calcium in eukaryotes not only in the circuitry of metabolites (Fig. 4), but also in the links to tension (filaments), local signalling (endoplasmic reticulum, ER), energy production (mitochondria and chloroplasts), the nucleus via phosphorylation, and to exocytosis (vesicles). Note that the flow is at a constant low level due to channels and pumps but can be augmented by extracellular events – triggering.

energy have to be active at all times to maintain the cell steady state as well as recovery from triggering.

Now we can turn back from the sources of energy to the eukaryote to the energy distribution in the cytoplasm itself after the incorporation of mitochondria and chloroplasts, i.e. to advanced aerobic single cell eukaryotes, and their activities. We need to see that the eukaryote cell not only has internal balance as in prokaryotes and a new balanced connection to internal energy but also has a homeostatic steady-state mechanism relating to the environment as well as triggered response to change. We believe calcium currents play a major role in this management. These currents have local intensity under differential controls in different parts of cells [7,8] but it must be remembered that of necessity flow is constant at a low level even in the non-triggered state (see Fig. 3).

## 5. Cytoplasmic activity of aerobic single-cell eukaryotes

The major effort in calcium research has been on structure/function relationships of the individual receptor proteins in eukaryotes (especially multi-cellular) when calcium enters a cell after a message is received. The calcium message is often amplified by either $IP_3$- or $Ca^{2+}$-stimulated release of calcium from ER stores. The calcium triggers all kinds of activity of kinases, phosphatases, pumps, contraction etc. at $10^{-6}$ M internal concentration, much of which is linked to energy, ATP, in a circuit. However, as sophistication of compartments grew the responses were required to have very different time constants. For example, the contractile response (movement away from danger or toward a target) must be faster than internal metabolic rate change which must be faster than gene (nuclear) response. Thus action energy including distribution has to be directed disproportionately in time (Table 3) [3,4]. Here then there are required to be fast and slow calcium responses, which must depend on the way conformational changes of calcium proteins are managed. The manipulation of a calcium receptor protein is dependent upon exchange rates between calcium free and bound states to any other unit e.g. another protein, a membrane or phosphate compounds (see [3]). The responses are localised in space and not general to the whole cell, e.g. in amoeboid attachments, or T-junction muscle responses (Fig. 6). We have described the graded time scales of energy application to events in previous publications ([1] and see [4]). We have now noted above how calcium aids energy recovery through the organelles. The response can only be of intermediate speed. However, the size of the calcium input is also important. Thus, excess calcium sets in an additional wave of effects and assists apoptosis, degradation by proteases. Every cell then has a timed and graded set of responses and we may compare these times with the timing of calcium removal whether by $Ca^{2+}$ pumps (ATPases) or by $Na^+/Ca^{2+}$ exchange. The pumps are themselves activated by calcium. Notice how slower recovery systems are related to $Na^+/Ca^{2+}$ exchange and faster systems to $Ca^{2+}$ ATPases. The parts of the calcium circuitry has different time sequences much as can be devised in electronics.

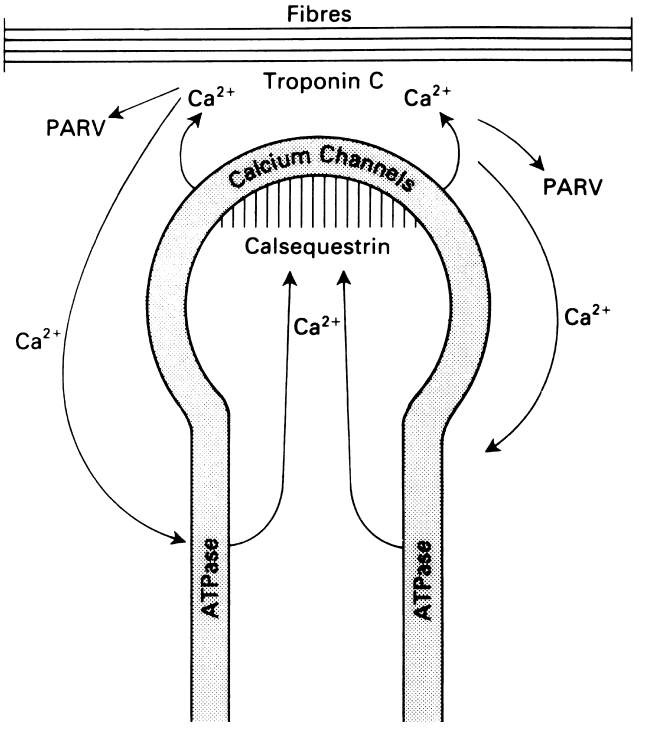


Fig. 6. The release system for calcium at the tip of a sarcoplasmic reticulum of a muscle cell. The calcium channels are placed very close to the contractile fibres and here calcium is stored in a calcium binding protein, calsequestrin. The calcium is removed by parvalbumin, PARV, and then by pumps (ATPase). The whole organisation has a weak current flow which can be stimulated by a nerve of chemical pulse.

Now all this activity (Table 4) can also be related

Table 4
Components of the fixed intracellular calcium current in eukaryotes

| Component | Function |
|---|---|
| $Ca^{2+}$ ATPase pumps, $Na^+/Ca^{2+}$ exchangers | Removing calcium from the cytoplasm to external fluids, vacuoles or organelles |
| $Ca^{2+}$ channels | Input of calcium from external fluids or vesicles to cytoplasm |
| Actinomyosin filaments | Maintenance of cell tension |
| Enzymes | Activation by calmodulins, annexins, S100 and so on, of kinases, phosphatases, proteases and so on (relayed to nuclear activity) |
| Proteins in vesicles | Calsiquestrin and others as storage of large amounts of calcium, internal condensers |

NB. All these systems are constantly active at a low level at rest. Activity general or selective causes changes in the calcium current in the cell stimulated by external events.

to triggering and its recovery to steady states but we have to see that this does not solve a major problem of action which has to be against the background of homeostasis. Homeostasis is based upon the resting or developmental level of flow as well as concentration in a cell which, for a single eukaryote cell, has to be maintained against a variable environment. It is here that it appears that calcium is the major communication connector. A nice example of the local circuitry is given next.

Recently the structure of the calcium reservoir in the endoplasmic and sarcoplasmic reticulum has been uncovered [9]. There is in both vesicular systems a protein, calreticulin or calsequestrin, which can hold some 40 $Ca^{2+}$ ions in a cleft of two thioredoxin-like domains. The proteins are present in almost crystalline array and are very close to both channels for calcium release and pumps for calcium uptake. The positioning of all three proteins (see Fig. 5) allows small circulating calcium currents at all times as well as quick release and recovery of the homeostatic balance. These *local current* flows are of extreme importance in the management of the cell since the calcium currents are in communication with filaments as in a muscle cell and maintain cell metabolism through kinases, cell shape through filament ATPases, and ion levels through plasma membrane pumps. There are also $2Na^+/Ca^{2+}$ exchangers in some of these membranes and perhaps associated with organelles and energy flow homeostasis. This general local current is reminiscent of that of the protons in mitochondria and chloroplasts. It is also reminiscent of advanced computer circuitry where a low level of flow is maintained at all times.

Here we draw attention to the new knowledge concerning mitochondria, chloroplasts and reticula. In early work electron microscopy apparently showed that these compartments were small, often drawn as almost spherical vesicles, with many vesicles of each kind in a cell, e.g. calsizomes for calcium storage. In the last few years serial electron microscopy has shown that this is a false picture. In fact all three vesicular structures consist of very large often single bodies occupying a considerable volume but composed of waving tubular or laminar membranes. The change in the knowledge of these structures requires a re-thinking of the way in which they work. Instead of treatments which deal with individual small spherical vesicles, with generalised bulk gradients of ions and potentials, we need localised models of activity [10]. Thinking in terms of steady-state vesicular thermodynamics, for example as characterised by chemi-osmosis, may well miss the sophistication permitted in these large systems, due to localised responses which are so clearly seen by calcium indicators [11], and which will affect localised mitochondrion proton activity.

## 6. The further needs of a homeostasis in multi-cellular organism

A multi-cellular organism has additional requirements to those of a single eukaryote cell. It must have:

1. An extracellular matrix to hold separate cells in place in organs yet to allow growth this system must be adjusted using energy
2. An extracellular fluid for transfer of material which has to be in a protected container, skin, cuticle or shell, and circulated using energy
3. Messenger systems to give information from receptors in one area (e.g. the head) to all other areas. Again there is an energy cost.

Note that as well as being used in responsive changes the above must act in the maintenance of the steady state of cell-cell and organ-organ relationships. This implies a constant current flow of many ions and molecules throughout the organism. These needs are apart from the requirements for material and energy flow in single cells which we have just described. No synthesis occurs outside cells and no ATP energy is available there so that whereas there are the sources of energy in the cytoplasm – chemical bond energies (ATP), tension, and gradients – there are really only two sources of energy in the extracellular fluids – tension and gradients. Changing tension is supplied by contractile devices in muscles, heart and arteries for example – all calcium activated. The fact that the cell is a massive store of molecules both in the cytoplasm and in vesicles means that relative to the extracellular fluids the cell stores a multitude of energised organic chemical gradients especially in its vesicles. There are calcium-dependent release mechanisms for these gradients – exocytosis from vesicles – which allow the external fluid to carry messages, e.g. transmitters and hormones, or to transform the ejected proteins for use externally. Some of these proteins are for the synthesis of the extracellular matrix and some for protection, digestion, and so on. The message systems to maintain the workings of the whole are a variety of transmitters and hormones which are exposed to a fixed extracellular calcium. The activities of very many extracellular proteins as well as the exocytosis of hormones etc. depend on calcium as in the following example. (Note the reverse of cell activation where changing calcium adjusts fixed levels of enzymes making them active while in the extracellular fluids changing organic chemical levels are activated by fixed calcium.)

## 7. Exocytosis and signals

As stated there is no synthesis outside cells. Hence all the organic requirements for maintenance (signalling) and growth come from inside cells even if some of the basic units have to be transported into the cells from other cells. The demand is for a controlled steady level of messenger molecules in the circulating fluids released by cells as well as a controlled release of structural proteins and protective enzymes for extracellular use. Nothing is permanent in organisms. There has to be both steady-state flow and growth of agents which communicate to the inside from outside. We know that external events cause excitation and calcium entry but internal stress, which could be for example oscillating mechanical stretching using the extracellular filaments, may also allow calcium to enter the cell. Both may stimulate regular vesicle exocytosis so that calcium is a maintenance and growth signal between cells assisted by specific and selective transmitters and hormones and excited proteins all of which circulate at certain levels. Do not forget that these hormones and proteins are released from vesicles but are constantly degraded so must be constantly distributed. Thus calcium governs growth and maintenance systems in that it governs release of *all* these substances by exocytosis. We have to recognise that the functioning of a large multi-cellular organism and equally for each single cell requires constant flow even at rest so that homeostasis is based on current flow, carefully controlled.

Now such mechanisms as these require very close management at all times of the calcium levels in the extracellular fluids, and simultaneously the control of the extracellular matrix itself. These controls operate over time scales of weeks, months and years and are quite unlike those used in the cytoplasm of single cells. Thus we have to consider new additions to

Table 5
Components of the extracellular calcium current in multi-cellular organisms

| Component | Function |
|---|---|
| Epithelial cells | Calcium input using asymmetrically placed pumps and channels and carrier proteins, calbindin |
| Kidney cells | Excretion of excess calcium |
| Bone and bone cells | Maintenance of calcium levels in the extracellular fluids |
| Muscle cells | Maintenance of flow in body fluids |
| Glands | Maintenance of circulating hormones to relay information about calcium levels to DNA protein synthesis |

NB. All these systems are constantly active at a low level at rest. Different hormone or ion current level ($Na^+/K^+$) changes can switch the calcium levels temporally. During development the calcium currents are changing continuously e.g. in fertilisation.

the calcium network since calcium in the extracellular fluid can only be fixed if it is replaced while it is constantly lost (see Table 5).

## 8. Extracellular fluid calcium

Calcium is such a major player in the maintenance of multi-cellular homeostasis that its extracellular concentration must be very finely managed. Here we meet an intricate network of interactions associated with mineral deposits especially in higher animals. What is required is regulated uptake and deposition of calcium itself at a steady rate so that resources are constant, keeping calcium flow in the whole extracellular system fixed. The deposition is of calcium in many structures outside of cells including minerals such as bone. The need is to take in calcium steadily while rejecting excess. Thus there are epithelial cells for uptake with specially placed calcium inlets and pumps for injection into the organism at different sides of these cells and calcium proteins for transport across their cytoplasm, for example calbindin. Excretion is via the kidney. This activity is regulated by the production of the appropriate proteins which means that there has to be feedback instruction to DNA from the circulating levels of particular units. One instruction is via the maintained levels of vitamin D generating a message to zinc-finger transcription factors. This sterol commands the synthesis of the calbindins and also the protein production for the control of calcium deposition in bone. Thus bone is maintained as a deposit of calcium and as far as this article is concerned bone is a condenser of the current carrying ion, calcium (Table 5).

Since the uptake of calcium is necessary to keep bone in a good condition a very small excess concentration over the solubility product is desirable since a large excess will lead to general precipitation. What we observe is that bone is constantly monitored by cells so that the calcium level is very precisely maintained at a precise effectively buffered level. A major protein is osteocalcin. The management of extracellular calcium current is emphasised by a second system.

Recently a second mode of management of extracellular calcium has been discovered [12]. The level of calcium in the extracellular fluid affects via G-proteins and $IP_3$ the level of cytoplasmic calcium inside certain cells. The rise of internal cytoplasmic calcium level with fall in external calcium causes release of parathyroid hormone from vesicles to the extracellular fluid where it acts to increase calcium release from bone. The functions of vitamin D and of the parathyroid hormone are complementary.

We see that the circuitry of extracellular calcium is linked to the circuitry of intracellular calcium in many ways making the total circuit network very complex.

## 9. Brain growth: a special calcium system

The brain is a peculiar organ in that it develops (grows) to the dictates of the environment as perceived by the animal but not under genetic control. Thus it is now experience which makes growth and synapse connection. The initial event is a $Na^+/K^+$ current which releases organic chemicals from vesicles of nerves to stimulate activate growth connections. However, it is again modulation of calcium flow which controls release and synapse growth [8,13]. There is an extraordinary feature in that the extracellular fluid is now quite different in calcium concentration from that of the whole body since the brain is immersed in cerebrospinal fluids (CSF). For how long has this been true? Why is it necessary? Why is CSF low in calcium? It needs energy to keep it so. One possibility concerns the sensitivity of glutamate release to the levels of external calcium [13]. Much as is the case for parathyroid hormone release, external calcium concentration affects glutamate release. Another possibility is that the low calcium is an insurance against precipitation of calcium carbonate, as in the ear, or of calcium phosphate, or against any calcium sensitive reactions such as clotting of proteins. However, much of the operation of the brain represents another separate calcium-linked circuit.

## 10. Conclusions

This article has as its objectives the demonstration that energy dissipation as well as material transport

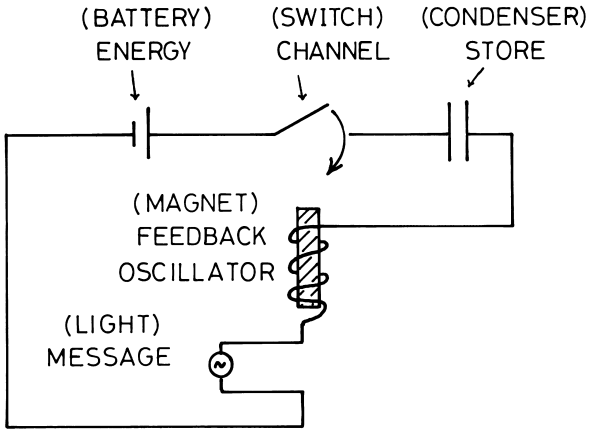


Fig. 7. A much simplified relationship between the circuits of electrons in man's electronic devices and the electrolytic circuits of calcium in cells and organisms. The level of comparison extends to the brain and senses in that triggering of both electron and calcium circuits are similar. Energy: $Ca^{2+}$ battery storage cell; Switches: $Ca^{2+}$ channel openings; Condensers: vesicles, ER etc., (bone); Device: protein receptors; Connectors: $Ca^{2+}$ transfer through membrane pumps.

across membranes is more often that not calcium related. The connection is made in several ways:

1. Direct control over ATP production by organelles
2. Influx of $Ca^{2+}$ to cytoplasm as a general signal
3. Efflux of many proteins and other molecules to the high calcium of extracellular fluids
4. Control by $Ca^{2+}$ over contractile poise
5. Control by $Ca^{2+}$ over the other gradients of elements e.g. links to $Na^{+}$, $K^{+}$ etc.
6. Whole body movement of calcium between organs.

$Ca^{2+}$ is certainly a fast trigger through its influx but it can also create a slow sustained response. However, it is linked to so many other cellular features that its additional role could well be in general homeostasis of the resting state. If this is so each cell and each organism has to be looked at as if it were an electrolytic device parallel to an electronic system, e.g. a computer (Fig. 7). If we look at cells as we look at modern computers then the equivalent devices have to be found. The equivalent of the battery is the storage of calcium in gradients, water replaces wires, switches are current (calcium) induced conformation changes, condensers are vesicles (ER etc.) inside the cell, retaining calcium, or bone. As in a computer (compare especially the brain) there is constant low-level current flow in the maintained state. The implications is the calcium ions constantly monitor and maintain homeostasis as well as acting as a trigger. The parallel is between an electron and the calcium ion. If this is accepted then we can not understand calcium function except in terms of integrated circuits.

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