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The Sodium Pump in the Evolution of Animal Cells

Wilfred D. Stein

Phil. Trans. R. Soc. Lond. B 1995 **349**, 263-269
doi: 10.1098/rstb.1995.0112

The sodium pump in the evolution of animal cells

WILFRED D. STEIN

Biological Chemistry, Silberman Institute of Life Sciences, Hebrew University, Jerusalem 91904, Israel

SUMMARY

Plant cells and bacterial cells are surrounded by a massive cellulose wall, which constrains their high internal osmotic pressure (tens of atmospheres). Animal cells, in contrast, are in osmotic equilibrium with their environment, have no restraining surround, can take on a variety of shapes and change these from moment to moment. This osmotic balance is achieved by the action of the energy-consuming sodium pump, one of the P-type ATPase transport protein family, members of which are indeed also found in bacteria. The pump's action brings about a transmembranal electrochemical gradient of sodium ions, harnessed in a range of transport systems that couple the dissipation of this gradient to establishing a gradient of the coupled substrate. The primary role of the sodium pump as a regulator of cell volume has evolved to provide the basis for an enormous variety of physiological functions.

1. THE OSMOTIC PROBLEM OF A CELL IN A MARINE ENVIRONMENT

Plants are sessile; animals move. Plants trap the sun's energy to run their life processes; animals consume these plants and hence their stored energy, either directly or at some distance up the food chain. Plant cells are surrounded by a massive cellulose wall; animal cells are not. Animal cell membranes possess the sodium–potassium pump; plant cell membranes do not. Is there some connection between these facts? Clearly, animals move from plant to plant (or from preyed object to preyed object) to harvest stored energy. It seems reasonable that animals are able to move because their flexible cells lack the burden of the cellulose walls that constrain the bacterial or plant cells. What then is the connection between the presence of the sodium pump and the absence of the cellulose wall?

August Krogh first pointed out this connection in a paper in the *Proceedings* of this Society (Krogh 1946), an intuition later extended by Maizels (1954), Wilson (1954) and Leaf (1956). Krogh saw that *all* cells in an aqueous environment are under permanent threat of flooding because they contain non-permeable matter (nucleic acids, proteins, intermediary metabolites, energy stores), yet are immersed in a medium containing ions to which their membranes are, at least to some extent, permeable. In the absence of counter-vailing forces, the ions will tend to reach an equal concentration within and without the cell, leaving an osmotic burden within it. Bacterial and plant cells parry this threat of death-by-flooding by enclosing each cell in a restraining cellulose wall, able to withstand the large osmotic pressures that develop. Such cells are then condemned to a largely sessile life. Animal cells have evolved along another path. They possess a membrane-located pump that pumps one of the major ions, sodium, out of the cell. The sodium pump, in essence, immobilizes sodium externally and, as a result, the cell can be in osmotic equilibrium and the osmotic effect of its impermeable metabolites is compensated for by the absence of intracellular sodium. How is this achieved and what evolutionary consequences follow?

2. THE EVOLUTION OF THE SODIUM PUMP

The sodium pump is a membrane-bound ATPase (Glynn 1993). It splits the energy-storing adenosine triphosphate (ATP) to yield adenosine diphosphate (ADP), releasing inorganic phosphate, and in the process moves three sodium ions out of the cell in exchange for two potassium ions. Sodium pumps from many animal cells have been cloned and sequenced (figure 1). The molecule is composed of two chains, the larger possessing many hydrophobic sequences presumed to be transmembranal, the smaller a single such sequence (figure 1). Much is known about the details

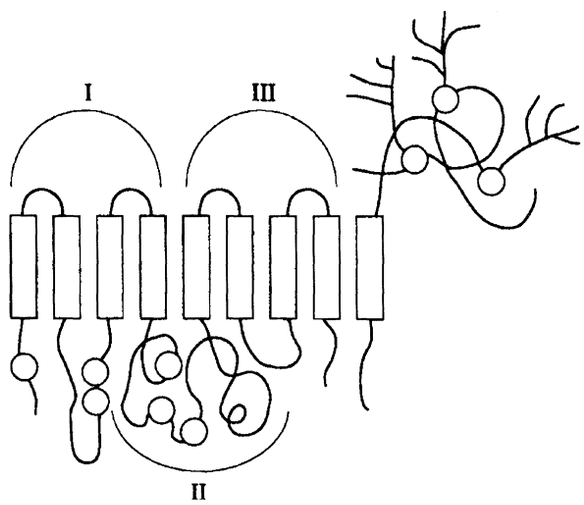

Figure 1. Schematic diagram of the sodium pump. The rectangles represent putative transmembrane sequences. Sections labelled I, II and III represent the N-terminal, cytoplasmic and C-terminal thirds of the molecule that were used for building the phylogenetic trees of figure 2*a*.

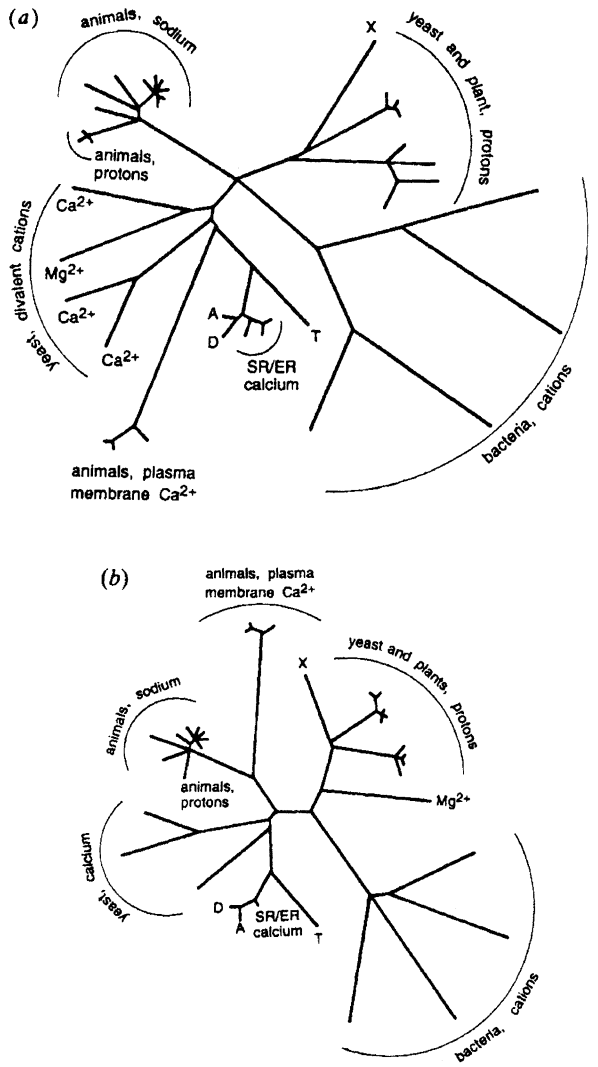

Figure 2. Unrooted phylogenetic trees for the P-type ATPases: (*a*) based on sequences in the N-terminal third of the molecules, labelled I in figure 1; (*b*) based on the cytoplasmic loop labelled II in figure 1. Mg^{2+} represents a Mg-activated ATPase from *Salmonella typhimurium*. A, D and T are the calcium pumps from *Artemia salina*, *Drosophila melanogaster* and *Trypanosoma brucei* respectively, while X is a cation pump of unknown specificity from *Leishmania donovani*. (Redrawn from Fagan & Saier (1994).)

of its action but how ATP splitting is coupled to ion movements is not yet clear (Glynn 1993). What *is* known is that, in the presence of sodium, phosphate is transferred from ATP to the pump enzyme itself before being liberated into the cell under the influence of potassium.

There is a superfamily of such membrane-bound ATPases that are phosphorylated during the course of their action. Some require sodium and potassium as cofactors. In others the sodium is replaced by hydrogen ions, in still others by calcium, while the potassium can be replaced by protons. Many of these so-called P-type ATPases have been cloned and sequenced. With one trivial exception, no protein other than a P-type ATPase exhibits significant sequence similarity with this superfamily. Fagan & Saier (1994) have analysed 47 of these sequences and constructed the pertinent phylogenetic trees. Figure 2 shows two possible phylogenetic trees. Figure 2*a* is based on the sequences of the first section of the molecule, containing the N-terminal transmembrane sequences, figure 2*b* on the cytoplasmic loop of the pump that contains the ATP-binding (and phosphorylated) sequences.

The two trees are concordant although not identical. In both, four major families exist. One family includes four of the bacterial P-ATPases, pumping potassium, cadmium, copper or an as yet unknown cation, respectively. More or less equally closely rooted to this family are two large families. One includes the calcium-stimulated ATPase of yeast and other protozoa and of the endoplasmic reticulum and the sarcoplasmic reticulum of many animals. (In the tree of figure 2*a*, the calcium ATPases from the plasma membrane of the animal cells cluster together with the remaining calcium ATPases, but in the tree of figure 2*b* they cluster separately.) The second large family contains the proton pumps of plants, yeast and fungi. A fifth bacterial P-ATPase, a Mg^{2+}-dependent ATPase from *Salmonella typhimurium*, clusters with the eukaryotic calcium ATPases in figure 2*a* but with their proton ATPases in figure 2*b*. This suggests that the split between the remaining four bacterial ATPases and all other members of the P-ATase family occurred *before* the appearance of the eukaryotes. Well separated from the proton and the calcium pumps is the fourth family, containing the sodium pumps (from hydra to *Drosophila* to mouse to man) and also the gastric and colon proton pumps. To the extent that one can use such unrooted phylogenetic trees to uncover evolutionary relations, one might hazard that the bacterial cation P-type ATPase pumps are primitive. After a duplication event, of which the *S. typhimurium* Mg^{2+}-ATPase is a survivor, and from this branch, evolved the calcium pumps of the intracellular vesicles and the plant plasma membrane proton pumps, early and separately in eukaryotic evolution. From such vesicular calcium pumps evolved the plasma membrane calcium pumps and (perhaps from them; figure 2*b*) the sodium pumps, and from these the gastric and colon proton pumps.

3. DONNAN AND DOUBLE DONNAN DISTRIBUTIONS

Figure 2 confirms Krogh's intuition (Krogh 1946) that the sodium pump may have evolved when the animals split off as a separate eukaryotic kingdom. I now show how the possession of a sodium pump enables the animal cell to control its volume. First, notice that many of those intracellular components that constitute the osmotic load are negatively charged. (Consider, for example, the nucleic acids and the intermediates of the tricarboxylic cycle and of glycolysis.) Now, there can be no substantial excess of charge within a closed phase such as a cell. The impermeability of the fixed intracellular anions thus brings about a Donnan effect such that mobile anions are excluded from the cell and mobile cations concentrated within it. Thus, a cell immersed in sea water, with no sodium pump present, would accumulate intracellular sodium far above that present extracellularly, to an extent determined by the ratio of extracellular anion to intracellular fixed anion.

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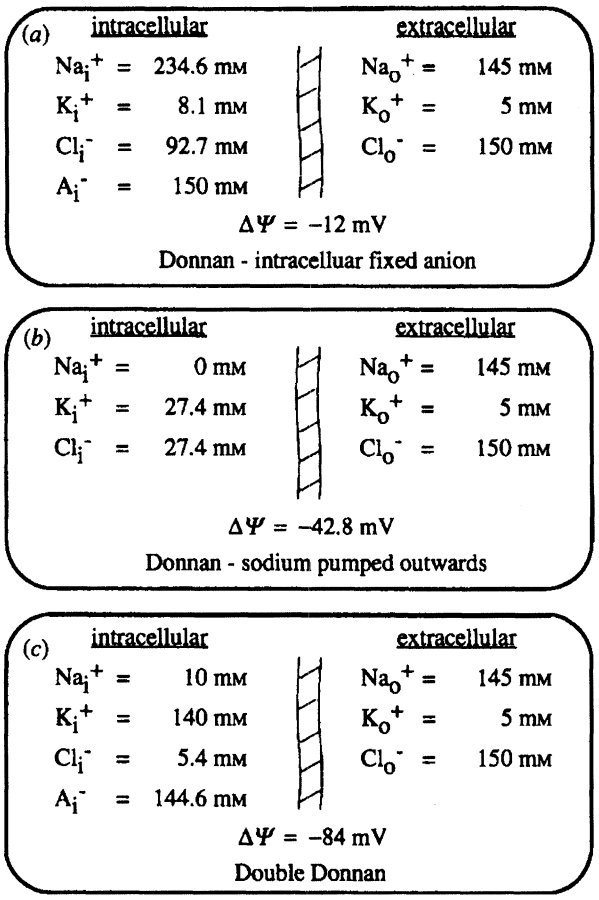

Figure 3. Ionic distributions across cell membranes at charge equality, with: (*a*) a fixed anion present intracellularly; (*b*) the sodium pump maintaining zero sodium within the cell; (*c*) both a fixed intracellular anion and an active sodium pump present, the double Donnan distribution.

Figure 3*a* depicts this situation at the steady state with no excess of charge within the cell. The cell develops an electric potential across the membrane, negative inside, owing to the immobile intracellular anions. The distribution of mobile ions given by this Donnan potential is $[Na]_{in}/[Na]_{out} = [K]_{in}/[K]_{out} = [Cl]_{out}/[Cl]_{in} = \exp(\Delta F/RT)$, where Δ is the potential across the membrane, F the Faraday constant, R the gas constant and T the absolute temperature. The cell is in charge balance but not in osmotic equilibrium, owing to the excess of total ions within the cell. The cell will swell indefinitely. Figure 3*b* depicts the opposite case. There is now no fixed anion, but a pump ensures that sodium ions are kept out of the cell, bringing about an excess of fixed cation outside the cell and hence a Donnan potential. Again, at steady state, the mobile ions distribute themselves in accordance with the Donnan potential. Again there is charge balance, but now an excess of osmotic material *outside* the cell. The cell will *shrink* indefinitely.

Finally, figure 3*c* depicts what might be found in a typical animal cell. Here, a fixed anion imposes an osmotic stress inwards and the sodium pump balances this with an osmotic stress outwards. This 'double Donnan' allows the cell to be in osmotic equilibrium as well as in charge equality, at the expense of energy consumed by the sodium pump's ATPase. Mobile ions distribute themselves according to this double Donnan, the size of the Donnan potential being given by the combined effect of the fixed anion and the sodium pump. As an example, with internal anion fixed at 144.6 mM, charge and osmotic equality are achieved if internal sodium is set at 10 mM, when the Donnan potential becomes 84 mV, the Donnan ratio 28, internal chloride 5.4 mM and internal potassium 140 mM.

These computed values should be compared with those found for different animal cells, listed in table 1. Also listed are the measured resting membrane potentials for each cell and, for each ion, its Nernst potential, that expected were the ions' distribution to be determined only by this membrane potential. Sodium concentrations are everywhere low, far from those expected for a passive distribution, not surprisingly since all these cells contain the energy-consuming sodium pump. For the excitable cells, the measured intracellular potassium and chloride ion concentrations are not far from the computed values and the double Donnan model gives an excellent

Table 1. *Measured and predicted ion distributions in some animal cells*

	[Na]/mM			[K].mM			[Cl]/mM				
cell type	out	in	Nernst	out	in	Nernst	out	in	Nernst	$\Delta\Psi$/mV	ref.
hydra, body	46	17	+25	0.2	51	−140	6.5	12	+15	−40	a
Anodonta mantle	21	14	+10	0.7	28	−93	15	10	−10	−31	b
Sepia axon	486	77	+46	10	267	−83	560	41	−66	−60	c
Rana axon	120	10	+63	2.5	130	−100	121	3.5	−89	−93	d
mammalian skeletal muscle	145	12	+67	4	155	−98	123	4.2	−90	−90	e
Ehrlich ascites	160	26	+49	4	134	−93	157	51	−30	−15	d
L-cells	137	9	+74	5.7	167	−90	147	70	−20	−15	d
Amphiuma RBC	100	16	+49	3.5	155	−101	120	80	−11	−19	d
human RBC	164	13	+69	5	136	−85	154	83	−17	−9	d
lymphocyte	140	37	+36	5	165	−98	145	80	−16	−55	f
monocyte	140	21	+51	5	122	−85	145	104	−9	−35	g
neutrophil	140	25	+49	5	120	−85	145	103	−10	−59	h

References. a, Zeuthen (1992); b, Coimbra *et al.* (1988); c, Sjodin (1984); d, Altman & Katz (1985); e, Wilson (1979); f, Garcia-Soto & Grinstein (1990); g, Ince *et al.* (1987); h, Simchowitz *et al.* (1982).

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account of the ion distributions. We should note that nerve cells are of course highly specialized to maintain (and then manipulate) their membrane potential. In contrast, for some cell types commonly cultivated in the laboratory and also for erythrocytes, the potassium concentrations are very much higher (the calculated Nernst potential far more negative) than predicted from a passive distribution, based only balancing the fixed anion. For leukocytes, it is the chloride ions whose internal concentrations are higher than expected from passive diffusion. Can we account for these discrepancies?

4. THE POST–JOLLY EQUATION RELATING CELL VOLUME, CELL CONTENT AND THE PUMP–LEAK RATIO

The Post–Jolly equation links the transport properties of cells with their equilibrium volume (Post & Jolly 1957). Consider first a simple case with only two solutes present, neither being charged (figure 4, left). The cell, suspended in a medium containing only a permeant molecule at concentration $[P]_e$, contains an impermeant molecule at concentration $[A]_i$ and the permeant molecule at concentration $[P]_i$. At osmotic equilibrium

$$[A]_i + [P]_i = [P]_e. \quad (1)$$

A pump expels P across the cell membrane with rate constants k_p, while P leaks in and out with leak permeability constant, k_l. At equilibrium, efflux of P (pump + leak) equals influx (leak only), so that

$$k_l [P]_e = k_l [P]_i + k_p [P]_i. \quad (2)$$

By solving for $[A]_i$

$$[A]_i = [P]_e/(1 + k_l/k_p). \quad (3)$$

Thus $[A]_i$ depends on $[P]_e$ and the *leak/pump ratio* k_l/k_p. Now $[A]_i$ is simply the *amount* of internal impermeant material, X_i, divided by the equilibrium cell volume, V. Thus $[A]_i = X_i/V$. Substitution in equation (3) and rearrangement gives the Post–Jolly equation,

$$V = (X_i/[P]_e)\,(1 + k_l/k_p). \quad (4)$$

Thus, the volume at equilibrium of an osmotically responsive cell is determined by the amount of impermeant material X_i, the concentration of extracellular material $[P]_e$ and the leak/pump ratio k_l/k_p, and by these three parameters alone. Of these values, $[P]_e$ is a given datum of the natural world and is outside the control of the cell and X_i is fixed by the biological requirements of the cell, i.e. how much impermeant matter (enzymes, metabolites etc.) it contains. The leak/pump ratio must then be selected for, to determine an appropriate cellular volume.

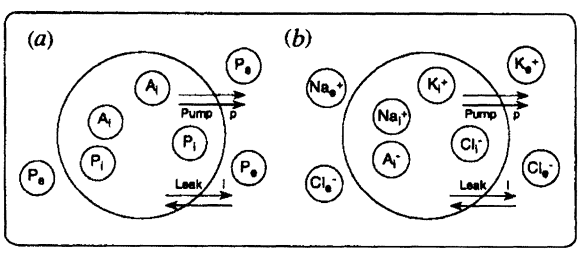

Figure 4. Pump–leak relations in schematic cells. (*a*) The scheme for uncharged species. P is permeable, A non-permeable. (*b*) The scheme for charged species. A is an impermeable anion. Subscripts i and e denote intracellular and extracellular components respectively; p is the pump's rate constant and l the leak rate constant.

In a more realistic model (figure 3, right), the cell contains two cations (Na^+ and K^+), only one of which is pumped out of the cell, an impermeant anion (A^-) and a permeant anion (Cl^-). For osmotic equilibrium, a Donnan relation for the permeant species and chemical equilibrium, as Na^+ is pumped out and leaks back, solution of the relevant equations gives

$$V = (X_i/[Na^+]_e)\,(1 + k_l/k_p), \quad (5)$$

equivalent to (4), with the extracellular concentration of pumped ion replacing that of the pumped molecule. The Donnan condition gives

$$[Cl^-]_i/[Cl^-]_e = [K^+]_e/[K^+]_i = r. \quad (6)$$

These equations give a complete description of the forces that control cell volume. The amount of impermeant intracellular matter $[X^-]_i$, the leak/pump ratio of pumped-out ion (k_l/k_p) and the concentration of the pumped-out ion in the extracellular fluid $[Na^+]_e$ (this *not* being under control) determine the cell volume. The cell volume in turn determines the *concentration* of impermeant anion, hence the Donnan ratio, and hence the equilibrium concentrations of all non-pumped ions. The results are remarkably general, yet simple.

But what determines the leak/pump ratio in the resting cell or in a cell subjected to acute changes in cell volume? Any mechanism for the regulation of cell volume requires that the cell can measure its own volume. We do not know how this is done (Parker 1993) nor how the cell has achieved the balance of volume and leak/pump ratios that determine long-term volume regulation. We know much, however, about how the leak/pump ratio is controlled in short-term responses.

5. THE THREE CLASSES OF ION DISTRIBUTION RATIOS

Inspection of table 1 reveals a major fact not accounted for by this naive sodium pump model: for all these cells, the potassium ion concentrations are high, often far higher than expected from the simple double Donnan model. Indeed, nearly all cells (red blood cells of cats and dogs being two of the exceptions) contain potassium ions as their major cation. This is true for animal, plant and bacterial cells, so that the requirement for high intracellular potassium seems as old as the RNA world itself (an intriguing fact with no accepted explanation; but see Herzberg (1974)). Recall that the sodium pump of animal cells is not merely a pump but also a sodium–potassium exchanger, bringing potassium into the cell in exchange for the sodium pumped out. This produces the accumulation of intracellular potassium, a function that bacterial and plant cells perform by potassium–

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proton exchange. If a high intracellular concentration of potassium is, indeed, a necessity for a nucleotide-based life, animals have linked this requirement to the sodium pump's maintenance of cell volume. The low sodium/high potassium intracellular ion ratio appears to be a property of the pump itself. At the intracellular face, the pump binds either sodium ions or potassium ions, the former with high affinity, the latter with low. Pumping continues until sufficient potassium has entered to compete with the reduced intracellular sodium. This regulation of the rate of sodium efflux enables the inward leak to be balanced, determining the ratio k_l/k_p in equation (5).

The cells listed in table 1 seem to fall into three main classes concerning their transmembrane ion distribution ratios. First are the excitable cells, nerve and muscle. Direct measurements show that the membranes of this class of cells possess channels for both potassium and chloride ions, with potassium ions crossing the resting membrane of the nerve some five times more rapidly. For such cells, a pump that exchanges potassium for the pumped sodium will generate an outward flow of KCl limited by the rate of chloride flow but still rapid. The cell will lose its internal osmotic balance and will shrink unless some counter measure is developed. Indeed, nerve cells possess, as a major contributor to the internal fixed anion, an unusual cell constituent, isethionic acid. This is an impermeable anion whose role appears to be to ensure that the cell's volume can be maintained in the face of a high negative resting membrane potential and a high permeability to both potassium and chloride ions. For this first cell class, the ionic and volume interrelations are well described by equation (5), with volume being maintained in the long and short term by manipulating the concentration of the internal fixed anions (Law 1994).

The second class includes those cells (red blood cells and tumour cells) for which the chloride distribution ratio is still close to that determined by the membrane potential, itself computable from the simple double Donnan model and equation (5). For these cells, however, the intracellular potassium concentration is not that given by the membrane potential. Potassium is kept high internally by the action of the sodium pump, satisfying a requirement that is even more basic than volume regulation. Exchange of potassium for sodium does not alter the osmotic relations across the cell nor the charge distribution and hence the double Donnan potential. In these the membrane's resting permeability to potassium is low and that to chloride higher, and they maintain a high intracellular potassium level economically in the face of an electrochemical potential driving potassium outwards.

The third class includes the mammalian white blood cells. Here, the cell membrane possesses a high permeability for potassium, a low permeability for chloride. The high intracellular potassium concentration, brought about by the sodium–potassium pump, is compatible with the transmembrane potential and, indeed, largely determines this potential. The intracellular chloride is far higher than that expected from the transmembrane potential, being maintained high by a set of coupled pumps linked, in the final analysis, to the sodium ion gradient (Macknight *et al.* 1994). Chloride is thus fixed intracellularly and contributes to the fixed anion of equation (5), actively maintaining the cell's volume, as isethionic acid does in the nerve cell.

6. VOLUME REGULATORY INCREASE AND VOLUME REGULATORY DECREASE

Using this classification of cells, which we see to be based on their ratio of potassium to chloride permeabilities, we can approach the regulation of cell volume. Consider again equation (5). Here, the cell volume is fixed in part by the ratio of leak/pump rate constants, k_l/k_p, for the pumped sodium ion. Of these rates, k_p, the pump's rate constant, seems poorly regulated in the short term in most animal cells. (The membrane's calcium pump is, in contrast, tightly regulated in the short term (Carafoli 1991).) Long-term regulation of the pump is achieved by altering the *number* of sodium pumps per unit surface area of the cell, i.e. by synthesis of more pumps (Gick & Ismail–Beigi 1990), a slow process generally taking hours, or by increased degradation of existing pump molecules. The leak of sodium occurs through membrane channels or through membrane carriers (Stein 1990). Excitable cells regulate their sodium fluxes through channels readily and rapidly (Stein 1990) but more typical cells regulate their sodium fluxes through cotransport or symport (Geck & Heinz 1986; Hoffmann & Ussing 1992). Here, a membrane-bound carrier moves sodium across the membrane together with chloride ions in a 1:1 stoichiometry or together with an additional potassium ion and *two* chloride ions. In both cases the product of the existing gradients of sodium, chloride and potassium (if transported by the carrier) at each face of the membrane constitutes a force driving sodium into the cell, opposed to the outwardly directed pump. This condition is fulfilled for all the cells in table 1. The cotransporter for sodium and chloride (with or without potassium) *is* tightly regulated, being activated when the cell's volume is *reduced* from normal, i.e. with the cell in a hyperosmotic milieu. On activating the cotransporter, sodium enters the cell, and water follows it osmotically, correcting the volume deficit. This is *volume regulatory increase*. At the 'correct' cell volume, the cotransporter is deactivated and the basal leak/pump ratio for sodium again determines the cell's volume. Sodium–chloride cotransport (with or without potassium) is found in many animal cells, being the major mechanism for regulation of cell volume in the direction of its increase.

If the cell is to regulate its volume *against* an increase (perhaps brought about by a temporary cessation of sodium pumping), downward regulation of the sodium leak will not suffice. The leak is already at a basal value. If the internal concentrations of potassium and chloride are those given by the Donnan potential (as in the excitable cells in table 1), these ions cannot contribute to an outward leak to compensate for a

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volume increase. They are already at equilibrium with no force driving them outwards. A volume decrease can be achieved only by downward regulation of the internal fixed anion concentration. However, for cells in which *neither* potassium nor chloride ions are at their electrochemical potential (classes 2 and 3, §5) other mechanisms exist. For those cell types for which the membrane's basal potassium permeability is low, a standing electrochemical gradient of potassium ion exists, outwardly directed. A controlled outward flow of K^+ can occur, as occasion demands, accompanied by osmotic water, causing a reduction in cell volume. This is one form of *regulatory volume decrease*. The upwardly regulated outflow of potassium ions can occur via a volume-sensitive membrane channel, when chloride follows to maintain charge balance, or it can occur via a cotransport of potassium ions with chloride ions, when it is the chemical gradient of the potassium/chloride product that is the driving force for efflux. Finally, in cells where the chloride permeability is low while the potassium ions are in electrochemical equilibrium across the membrane, an upward regulation of a potassium channel cannot bring about a potassium-driven conductive efflux. Upward regulation of a chloride channel can, however, lead to enhanced KCl efflux and hence to volume decrease (Hoffmann & Ussing 1992). In addition, the activation of a cotransport of potassium and chloride ions, which is *not* electrogenic and hence is not dependent on the membrane potential, can here again lead to enhanced ion efflux and volume decrease (Garcia-Soto & Grinstein 1990).

We identified at least two different mechanisms used to allow a sodium leak, the Na–Cl cotransporter and the Na–K–2Cl cotransporter. Similarly, several mechanisms exist for the potassium leak. This can be a K–Cl cotransporter or a K channel accompanied by a separate chloride pathway, or a chloride channel with potassium in a separate pathway. Figure 2 suggests that the sodium pump appeared only once in evolution, which implies, if the above arguments are correct, that the pump is the *primary* mechanism of volume regulation. In contrast, volume regulatory decrease and increase mechanisms have appeared several times, and are apparently fine-tuning mechanisms, superimposed on the basic sodium pump system.

Many additional cotransport and countertransport systems link the animal cell's transmembrane sodium (and/or potassium and chloride) gradients to the driving of a range of metabolites into and out of cells. These include the transporters for various amino acids, many sugars, protons, calcium ions, iodide, vitamins and other metabolites. These transporters form numerous evolutionarily related families (Saier 1994*a*, *b*) and are found in all phyla. Their action underlies much of cell physiology and of general physiology. In animal cells, most such systems are linked to the gradient of that ion, sodium, whose transmembrane concentrations are determined by the need to fix and to regulate cell volume. By freeing the animal cell from the constraint of an external wall, the evolution of the sodium pump allowed the cell subsequently to evolve a wealth of coupled transport systems, opening up new modes of existence compatible with an increasingly complex organization.

REFERENCES

Altman, P. L. & Katz, D. D. 1985 *Cell biology*, vol. I. Bethesda, Maryland: FASEB.

Carafoli, E. 1991 The calcium pumping ATPase of the plasma membrane. *A. Rev. Physiol.* **53**, 531.

Coimbra, J., Machado, J., Fernandes, P. L., Ferreira, H. G. & Ferreira, K. G. 1988 Electrophysiology of the mantle of *Anodonta cygnea*. *J. exp. Biol.* **140**, 65.

Fagan, M. J. & Saier, M. H. 1994 P-type ATPases of eukaryotes and bacteria: sequence comparisons and construction of phylogenetic trees. *J. molec. Evol.* **38**, 57.

Garcia-Soto, J. J. & Grinstein, S. 1990 Determinants of the transmembrane distribution of chloride in rat lymphocytes: role of Cl–HCO_3 exchange. *Am. J. Physiol.* **258**, C1108.

Geck, P. & Heinz, E. 1986 The Na–K–2Cl cotransport system. *J. Membrane Biol.* **91**, 91.

Gick, G. G. & Ismail-Beigi, F. 1990 Thyroid hormone induction of Na,K-ATPase and its mRNAs in a rat liver cell line. *Am. J. Physiol.* **258**, C544.

Glynn, I. M. 1993 All hands to the sodium pump. *J. Physiol., Lond.* **462**, 1.

Herzberg, M. 1974 Interactions between membrane functions and protein synthesis. *Eur. J. Biochem.* **45**, 161.

Hoffmann, E. K. & Ussing, H. H. 1992 Membrane mechanisms in volume regulation in vertebrate cells and epithelia. In *Membrane transport in biology*, vol. 5 (ed. J. A. Schafer, H. H. Ussing, P. Kristen & G. H. Giebisch) p. 317. Berlin: Springer-Verlag.

Ince, C., Thio, B., van Duijn, B., van Dissel, J. T., Ypey, D. L. & Leijh, P. C. 1987 Intracellular K^+, Na^+ and Cl^- concentrations and membrane potential in human monocytes. *Biochim. biophys. Acta* **905**, 195.

Krogh, A. 1946 The active and passive transport of inorganic ions through the surfaces of living cells and through living membranes generally. *Proc. R. Soc. Lond.* B **133**, 140.

Law, R. O. 1994 Taurine efflux and the regulation of cell volume in incubated slices of rat cerebral cortex. *Biochim. biophys. Acta* **1221**, 21.

Leaf, A. 1956 On the mechanism of fluid exchange of tissues *in vitro*. *Biochem. J.* **62**, 241.

Macknight, A. D. C., Gordon, L. G. M. & Purves, R. D. 1994 Problems in the understanding of cell volume regulation. *J. exp. Zool.* **268**, 80.

Maizels, M. 1954 Active cation transport in erythrocytes. *Symp. Soc. exp. Biol.* **8**, 202.

Parker, J. C. 1993 In defense of cell volume? *Am. J. Physiol.* **265**, C1191.

Post, R. L. & Jolly, P. C. 1957 The linkage of sodium, potassium, and ammonium active transport across the human erythrocyte membrane. *Biochim. biophys. Acta* **25**, 118.

Saier, M. H. 1994*a* Convergence and divergence in the evolution of transport proteins. *Bioessays* **16**, 23.

Saier, M. H. 1994*b*, Computer-aided analyses of transport protein sequences: gleaning evidence concerning function, structure, biogenesis, and evolution. *Microbiol. Rev.* **58**, 71.

Simchowitz, L., Spilberg, I. & De Weer, P. 1982 Sodium and potassium fluxes and membrane potential of human neutrophils: evidence for an electrogenic sodium pump. *J. gen. Physiol.* **79**, 453.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Sjodin, R. A. 1984 Contributions of electrogenic pumps to resting membrane potentials: the theory of electrogenic potentials. In *Electrogenic transport: fundamental principles and physiological implications* (ed. M. P. Blaustein & M. Lieberman), p. 105. New York: Raven Press.

Stein, W. D. 1990 *Channels, carriers and pumps*. San Diego: Academic Press.

Wilson, J. A. 1979 Principles of animal physiology, p. 230. New York: Macmillan.

Wilson, T. H. 1954 Ionic permeability and osmotic swelling of cells. *Science, Wash.* **120**, 104.

Zeuthen, T. 1992 From contractile vacuole to leaky epithelia. coupling between salt and water fluxes in biological membranes. *Biochim. biophys. Acta* **113**, 229.

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