# How could a cell regulate its internal $P_i$ concentration?

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Many mammalian cells appear to regulate their internal $P_i$ concentration against changes in extracellular $P_i$ [1]. Here we describe a theoretical model which shows how this may be partly accounted for by an active $P_i$ influx into the cell, balanced by a passive $P_i$ efflux.

In the absence of active transport, $P_i$ distributes passively in both directions across the plasma membrane so that the ratio ($R$) of intracellular concentration ($C_2$) to extracellular $P_i$ concentration ($C_1$) is equal to the passive distribution ratio $R'$. This depends on the transmembrane potential [2]. As this can be as much as 100 mV inside negative, $R'$ should be much less than 1. Observed values of $R$ may be several hundred times higher than $R'$ [3], so we shall initially ignore this passive contribution to $R$ and $C_2$.

Assume first that both the active influx ($I$) and the passive efflux ($E$) obey hyperbolic (Michaelis–Menten) kinetics: $Na^+$-linked influx does this in a number of cell types [4–8] but little is known about efflux. Assume first that $I$ is independent of $C_2$, because influx is thermodynamically irreversible (as it is in e.g. kidney [9]); and that $E$ is independent of $C_1$ for the same reason. Thus $I/I_m = 1/[1+(K_I/C_1)]$ and $E/E_m = 1/[1+(K_E/C_2]$, where $I_m$ and $E_m$ are maximum influx and efflux rates, and $K_I$ and $K_E$ are the extracellular and intracellular concentrations for half-maximal efflux and influx, respectively. At steady state $I=E$, and thus a relationship between $C_2$ and $C_1$ can be derived. Three cases need to be considered. If $E_m = I_m$, the plot of $C_2$ against $C_1$ is linear with slope $K_E/K_I$; and if $E_m < I_m$, the slope increases with increasing $C_1$. Neither of these resembles the regulation of $C_2$ with increasing $C_1$ that has been observed in intact cells [1, 3]. However, if $E_m > I_m$, $C_2$ has a hyperbolic form, approaching a maximum value $C_{2m} = K_E/[(E_m/I_m)-1]$ when $C_1$ is large. $C_2/C_{2m} = 1/[1+(K_c/C_1)]$, where $K_c$, the extracellular concentration for half-maximal cellular $P_i$, is $K_I/[1-(I_m/E_m)]$.

When is this regulation optimal? To quantify this, define $Z$ as the ratio of fractional change in $C_2$ to that in $C_1$, so $Z=(dC_2/C_2)/(dC_1/C_1)$. In the present case $Z=1/[1+(C_1/K_c)]$. At a given $C_1$, the best regulation is therefore achieved (i.e. $Z$ is smallest) when $K_c$ is smallest, i.e. when $E_m$ is large and $K_I$ is small. In the limit when $E_m$ is much greater than $I_m$ (i.e. efflux is approximately first-order with respect to $C_2$), $C_{2m}$ approaches $K_E(I_m/E_m)$ and $K_c$ decreases towards $K_I$, which is its minimum value. $K_I$ is thus an important limit on the efficiency of regulation of $C_2$.

If the passive equilibrium contribution to $R$ and $C_2$ cannot be neglected, the effective concentration of cellular $P_i$ which drives efflux is $C_2-(R'\cdot C_1)$, (rather than simply $C_2$). Therefore, at high values of $C_1$ the cellular $P_i$ concentration can continue to rise above $C_{2m}$ with slope $R'$, so $C_2 \approx C_{2m} + R'\cdot C_1$.

In this model, as discussed above, $K_c$ cannot be less than $K_I$. However, we have evidence that in cultured UMR 106 cells, $K_I$ for the Na-linked uptake mechanism is 0.3 mM [8], while apparent cellular $P_i$ is little reduced by incubation in the absence of $P_i$ [1], which implies that $K_c$ is very small. This discrepancy is unlikely to be fully explained by, e.g. non-cytosolic $P_i$ pools or artefactual generation of $P_i$ from some unsuspected highly labile organic phosphate pool during extraction or assay. We therefore propose a modification to the model (again neglecting the passive equilibrium contribution $R'$). Suppose the efflux process is co-operative, so that $E/E_m = 1/[1+(K_E/C_2)^n]$, where $n$ is the Hill coefficient. Then $C_2/C_m = 1/\{1+[(2^n-1)(K_c/C_1)]\}^{(1/n)}$, where $C_{2m}$ is now given by $C_{2m} = K_E/\{[(E_m/I_m)-1]^{(1/n)}\}$, and where $K_c$ is (as before) the value of $C_1$ at which $C_2 = (1/2)C_{2m}$, now given by $K_c = K_I/\{(2^n-1)[1-(I_m/E_m)]\}$. The effect of the sigmoid kinetics of $E$, superimposed on the hyperbolic kinetics of $I$, is to make the plot of $C_2$ against $C_1$ rise more rapidly towards $C_{2m}$ at low values of $C_1$. This arises from the fact that $K_c$ can now become less than $K_I$ (see above), and decreases with increasing $n$. Furthermore, $Z$ is now given by $(1/n)/\{1+[(C_1/K_c)/(2^m-1)]\}$, and decreases as $n$ increases. The overall effect of increasing $n$ is therefore tighter regulation of $C_2$ and elevation of $C_{2m}$.

However, it can also be shown that if influx shows co-operativity with respect to $P_i$, then regulation of cell $P_i$ is worsened, as $K_c$ (and $Z$ at any $P_i$ concentration) are increased.

Finally, if active influx is not completely irreversible (as assumed above), so that $I$ is decreased by increasing $C_2$, regulation of cell $P_i$ is worsened, as $K_c$ is increased.

In summary, a cell could partially regulate the intracellular concentration of $P_i$ (or any other membrane-transported species) in the face of variations in its extracellular concentration, simply as a result of the characteristics of its membrane transport mechanisms.

In addition to this simple 'open-loop' regulation, there are other possible levels of control. In the renal tubular epithelial cells, Na-linked uptake is increased ($I_m$ is increased, in the present notation) by incubation at low extracellular $P_i$ concentrations [5]. It is not clear (since these cells are specialized for transcellular $P_i$ transport) what effect this has on cellular $P_i$ concentration. However, a similar stimulation of maximum influx in creatine metabolism in cultured myoblasts is certainly reported to regulate the intracellular concentration of creatine [10]. Presumably such regulation requires a mechanism (as yet unknown) to sense either the extracellular concentration, or, more directly, the intracellular concentration of the transported species. The second of these would represent genuine closed-loop (feedback) control.

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