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Spatial gradients of cellular phospho-proteins

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Abstract If a protein is rapidly phosphorylated and dephosphorylated at separate cellular locations and protein diffusion is slow, then a spatial gradient of the phosphorylated form of the protein may develop within the cell. We have estimated the potential size of such gradients using measured values of protein diffusion coefficients and protein kinase and phosphatase activities. We analysed two different cellular geometries: (1) where the kinases is located on the plasma membrane of a spherical cell and the phospatase is distributed homogenously in the cytoplasm and (2) where the kinase is located on one planar membrane and the phosphatase on a second parallel planar membrane. The estimated gradients of phospho-proteins were potentially very large, which has important implications for cellular signalling.

Key words: Protein kinase; Phosphatase; Spatial localisation; Diffusion; Cellular gradient; Signal transduction

1. Introduction

Many cellular proteins undergo cycles of phosphorylation and dephosphorylation by protein kinases and phosphatases and this protein phosphorylation is one of the main ways by which protein activities are regulated in the cell [1]. Various protein kinases and phosphatases are localised to different components of the cell (e.g. the cell membrane, the cytosol, intracellular membranes or the nucleus) and may change their distribution in different conditions. The kinases and phosphatases of a particular protein are often located in different compartments. This suggests the possibility that there may be spatial gradients of the phosphorylated or unphosphorylated form of such proteins. For example, if the protein is phosphorylated at the cell membrane and slowly diffuses into the cytosol where it is dephosphorylated and the unphosphorylated form diffuses back to the membrane, then, there might be a spatial gradient of the phosphorylated form, high close to the membrane and low within the cell. Large cellular gradients of the phosphorylated or unphosphorylated form of proteins would have very important implications for cell signalling. We therefore estimated the size of such gradients using measured values of protein kinase and phosphatase activities and protein diffusion rates. Our estimates indicate that such gradients are very large.

2. Results and discussion

We first estimated the relative steady-state gradient for a protein that is phosphorylated by a kinase located exclusively on the cell membrane and dephosphorylated by a phosphatase located homogenously in the cell cytosol. If we assume that the phosphatase is not saturated by the target protein (a reasonable assumption for most phosphatases [2]), then, for a spherical cell, the relative difference in concentration of the phosphorylated form between the cell membrane and the centre of the cell is given by (Appendix A):

$$\frac{p(L)-p(0)}{p(0)} = \frac{\exp(\alpha L)-\exp(\alpha L)}{2\alpha L}-1; \quad \alpha = \sqrt{k_p/D} \qquad (1)$$

where L is the cell radius, $p(0)$ is the concentration of the phosphorylated form of the protein at the centre of the cell, $p(L)$ is the concentration at the surface of the cell membrane, k_p is the activity of the phosphatase and D is the diffusion coefficient of the protein in the cytosol (assumed to be equal in the phosphorylated and unphosphorylated forms). Note that the relative gradient is independent of the target protein concentration and kinase activity, whereas the absolute gradient and concentration of the phosphorylated form is not (Appendix A).

In order to estimate the relative gradient, all we need then is the radius of the cell (L), the diffusion coefficient of proteins in the cytosol (D) and the phosphatase activity (k_p). Eukaryotic cell radii vary from 5 to 50 μm [3], we use a radius of 10 μm here for illustrative purposes. The diffusion coefficient of soluble proteins in the cytoplasm has been estimated by a variety of methods to be between 10^{-7} and 10^{-8} cm^2/s [4–9], though it can be considerably lower if the protein reversibly binds to immobile components of the cell [8]. We will use a D value of 5×10^{-8} cm^2/s here for illustrative purposes. Phosphatase activities vary for different phosphatases, protein substrates and cell types. k_p is given by $k_{cat}\times[e]/K_m$. Typical values for protein phosphatase I are: k_{cat} 13 s^{-1} [2,10], K_m 0.04–10 μM [11,12], $[e]$ 0.5 μM (skeletal muscle) [13], giving a k_p between 162 and 0.65. Protein phosphatase 2B (calcineurin): k_{cat} 1–3 s^{-1}, K_m 2–20 μM, [14] 20 μM in brain and 1–4 μM in other tissues [15], giving a k_p of 1–30 s^{-1} for brain. Protein phosphatase 2C: k_{cat} 40 s^{-1}, K_m 0.6–8 μM, $[e]$ 20 nM [2], giving a k_p of 0.1–1.3. Protein tyrosine phosphatase 1B: k_{cat} 26 s^{-1}, K_m 0.1–0.3 μM, $[e]$ 0.01 μM [16,17], giving a k_p of 0.9–2.6 s^{-1}. Thus, k_p varies from roughly 0.1 to 100. With k_p values of 0.1, 1, 10 and 100, the relative concentration gradients given by Eq. 1 are 0.4, 9, 50 000 and 3×10^{17}, respectively. What this means is that for $k_p = 1$, there is an approximately 9-fold lower concentration of the phosphorylated form of the protein in the centre of the cell than at the cell membrane, i.e. the gradient is large. And for $k_p \gg 1$, there is no

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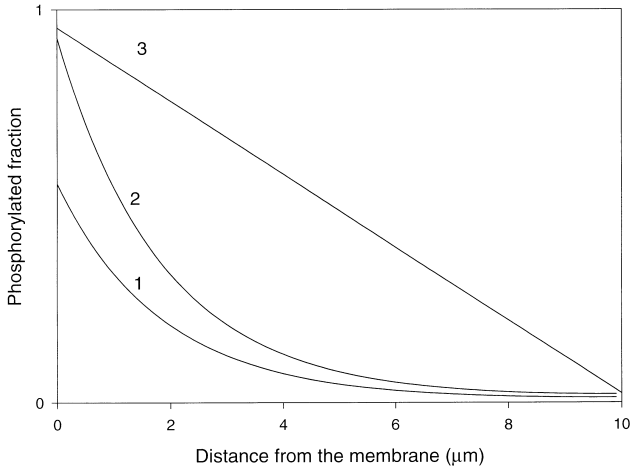

Fig. 1. Steady-state spatial distribution of phospho-proteins. Curves 1 and 2 show how the phosphorylated fraction decreases with the distance from the plasma membrane (where the protein is phosphorylated) into the cell interior (where it is dephosphorylated by homogeneously distributed phosphatase). The kinase activity is 1 (1) and 10 s^{-1} (2). The cell radius is 10 μm, the phosphatase activity $k_p = 2$ s^{-1}, the diffusion coefficient of the protein is 5×10^{-8} cm^2/s. Curve 3 corresponds to the case when the kinase is located at the cell membrane and the phosphatase is located at an internal membrane with a distance of 10 μm between these membranes. The parameter values are the same as for curve 1.

significant level of the phosphorylated form at the centre of the cell.

The absolute concentrations and gradients depend on the kinase activity, k_{kin}, in a Michaelis-Menten manner (Appendix A). Since the phosphorylated fraction falls off roughly exponentially as the distance from the membrane increases (Fig. 1, curves 1 and 2), the effect of k_{kin} changes is most significant for the phospho-protein concentration near the membrane. With $k_p = 1$ s^{-1} and k_{kin} values of 0.1, 1, 10 and 100 s^{-1}, the phosphorylated fractions near the surface of the cell membrane are 16, 66, 95 and 99%, respectively, of the total concentration of the target protein.

We have considered a number of other possible geometries of the distribution of the kinases and phosphatases, which in general lead to similar or larger relative gradients. For example, if the kinase and phosphatase are restricted to different membranes (e.g. kinase on the cell membrane and phosphatase on an internal membrane), then, the absolute gradients of the phosphorylated protein are, in general, larger and the relative gradients are less than those predicted above (Fig. 1, curve 3). The concentration of the phosphorylated form does not fall as precipitously as for a homogenous distribution of the phosphatases, but the difference in concentration at the same distance can be larger due to the higher concentration of the phosphorylated form near the cell membrane (Appendix B). However, obviously, if the kinase and phosphatase are located in the same compartment, then, the gradients disappear. The gradients calculated above are for the steady-state. Prior to the steady-state, the gradients are in general larger than for the steady-state.

We conclude that if the kinase and phosphatase of a protein are spatially separated in the cell, then large spatial gradients of the phospho-protein are inevitable (as long as the phosphatase activities are of the order of those given above). This has important implications for cell signalling. For example, the phosphorylated form of a protein phosphorylated at the membrane may be effectively restricted to a narrow domain below the membrane. Extremely complex spatial gradients of phospho-proteins may be expected in real cells with real (rather than spherical) geometries and non-uniform distributions of kinases and phosphatases. Kinases only stimulated on one side of the cell (e.g. in development, sensing of extracellular gradients or non-uniform attachment to other cells or substrates) will result in asymmetric distribution of phospho-proteins and dependent activities. Changes in the distribution of kinase or phosphatase will radically change these gradients. This may be one reason for these changes in distribution occurring in response to signals. Kinase cascades that are initiated at the membrane will result in overlapping gradients of the phospho-proteins that reach further into the cell at lower levels of the cascade, this may be one reason that cascades exist. Passing a message from the cell membrane to the nucleus would require specific geometric distributions of kinases, phosphatases or kinase cascades. Abrupt increases in kinase activity at the membrane (e.g. due to receptor stimulation) may result in waves of phospho-proteins and sustained oscillations of kinase activities in kinase cascades. Oscillations in kinase activity at the membrane may result in traveling waves of phospho-proteins in the cell. The analysis given here is for phospho-proteins but could equally apply to any reversible protein modification where the forward and reverse reactions are spatially separated in the cell, such as for G-proteins. All of these possibilities greatly increase the potential for signal processing by cells in the spatial and time dimensions.

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Appendix A

For a spherical cell, the following equation describes (radial) diffusion of the phosphorylated form (p) from the cell membrane (where p is produced) inside the cytosol (where p is dephosphorylated by homogeneously distributed phosphatase with the activity k_p),

$$\frac{\partial p}{\partial t} = \frac{D}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial p}{\partial r}\right) - k_p p$$

(e.g. Crank, J. (1975) The Mathematics of Diffusion, 2nd edn., Oxford University Press, London). The steady-state solution to this equation (taking into consideration that at the centre of the cell, there should be no diffusion flux, $\partial p/\partial r = 0$) reads:

$$p(r) = \frac{\text{const}\cdot(\exp(\alpha r) - \exp(-\alpha r))}{r};\ \alpha = \sqrt{k_p/D}.$$

Comparing p at $r = L$ and $r = 0$, respectively, Eq. 1 in the text is obtained. The constant factor in the solution above is determined by the activity of the kinase (k_{kin}) at the cell surface. If the kinase is far from saturation, then, this constant factor in $p(r)$ is given by,

$$\text{const} = \frac{ck_{kin}L^2}{(3D(\alpha-1/L)+k_{kin}L)\cdot\exp(\alpha L)+(3D(\alpha+1/L)-k_{kin}L)\cdot\exp(-\alpha L)},$$

where c is the sum of the concentrations of phosphorylated and dephosphorylated forms which is constant throughout the cell.

Appendix B

When the kinase and phospatase are located on different membranes and there is no phosphorylation/dephosphorylation activity between these membranes, the concentration of the phosphorylated protein decreases roughly linearly with the distance and the gradient is constant (assuming the membranes are so large that one-dimensional diffusion can be considered). If we assume that neither the kinase nor phosphatase is saturated by the target protein, then, the relative gradient is the following,

$$\frac{p(L)-p(0)}{p(0)} = \frac{k_p L^2}{D}$$

and the absolute gradient,

$$p(L)-p(0) = \frac{ck_p k_{kin} L^2}{D(k_p + k_{kin}) + k_p k_{kin} L^2}$$

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