Diffusion control of protein phosphorylation in signal transduction pathways

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Multiple signalling proteins are phosphorylated and dephosphorylated at separate cellular locations, which potentially causes spatial gradients of phospho-proteins within the cell. We have derived relationships that enable us to estimate the extent to which a protein kinase, a phosphatase and the diffusion of signalling proteins control the protein phosphorylation flux and the phospho-protein gradient. Two different cellular geometries were analysed: (1) the kinase is located on one planar membrane and the phosphatase on a second parallel planar membrane, and (2) the kinase is located on the plasma membrane of a spherical cell and the phosphatase is distributed homogeneously in the cytoplasm. We demonstrate that the control contribution of protein diffusion is potentially significant, given the measured rates for protein kinases, phosphatases and diffusion. If the distance between the membranes is 1 μm or greater, the control by diffusion can reach 33 % or more, with the rest of the control (67 %) shared by the kinase and the phosphatase. At distances of less than 0.1 μm, diffusion does not limit protein phosphorylation. For a spherical cell of radius 10 μm, a protein diffusion coefficient of 10^{-8} cm$^2 \cdot$ s^{-1} and rate constants for the kinase and the phosphatase of approx. 1 s^{-1}, control over the phosphorylation flux resides mainly with the phosphatase and protein diffusion, with approximately equal contributions of each of these. The ratio of phospho-protein concentrations at the cell membrane and the cell centre (the dynamic compartmentation of the phospho-protein) is shown to be controlled by the rates of the protein phosphatase and of diffusion. The kinase can contribute significantly to the control of the absolute value of the phospho-protein gradient.

Key words: Control Analysis, diffusion gradient, phosphatase, protein kinase, signal transduction cascade.

INTRODUCTION

Signal transduction within cells involves the phosphorylation of cytosolic proteins by membrane-associated kinases (e.g. see [1]). The phosphorylated (activated) forms of these proteins diffuse from the plasma membrane to cytoplasmic targets or to other cellular sites, and thereby transmit the signals within the cell. For instance, Raf, the initial kinase of the mitogen-activated protein kinase (MAPK) cascade, phosphorylates the downstream kinase, MEK (MAPK/ERK kinase, where ERK is extracellular-signal-regulated kinase), at the plasma membrane [2]. MEK then diffuses into the cytosol and phosphorylates the terminal MAPK, known as ERK. Activated ERK phosphorylates multiple cytoplasmic and nuclear targets (reviewed in [3,4]). The various phosphatases that generate unphosphorylated forms of proteins are often separated spatially from the respective kinases, e.g. delocalized over the cytosol or localized to different intracellular membranes, cytoskeletal structures or organelles. In fact, for our example, the MEK phosphatases are distributed throughout the cytosol [5,6]. The unphosphorylated forms of signalling proteins diffuse back to the plasma membrane, completing the cycle of phosphorylation and dephosphorylation.

It is instructive to examine what is controlling protein phosphorylation in a cascade, where the kinase and phosphatase are located at separate spatial locations. The control would include limitations on the steady-state phosphorylation and dephosphorylation rates, and the levels and subcellular distribution of phospho-proteins. Within an operational framework known as Metabolic Control Analysis (MCA) ([7,8]; reviewed in [9]), the control exerted by an enzyme or molecular process over the steady-state flux through a cascade or phospho-protein concentration can be quantified as the control coefficient. The control coefficient is defined as the fractional change in the steady-state flux or concentration divided by the fractional change in the activity of the enzyme or process (which caused that change), extrapolated to infinitesimally small change. This quantifies the ability of the enzyme or process, including non-enzymic reactions or diffusion, to influence the pathway rate and intermediate concentrations. Control analyses have recently been extended to cover signal transduction cascades [10,11]. However, the implications of spatial organization and diffusion for the control of protein phosphorylation have never been analysed quantitatively.

In metabolic pathways in which the enzymes are located in the same cellular compartment, e.g. glycolysis in the cytosol, the flux is not limited by metabolite diffusion [12]. By analogy with metabolic pathways, the phosphorylation of a protein in a phosphorylation cascade might also be controlled only by the kinases and phosphatases. However, in the present paper we demonstrate that the diffusion of signalling proteins may limit protein phosphorylation when the kinases and phosphatases are separated spatially within the cell. For signalling pathways, the pioneering work of Fell [13,14] predicted the potential existence of concentration gradients of cAMP in a cell in which adenylate cyclase is bound to the plasma membrane, whereas a high-K_m phosphodiesterase is located in the cytosol. We have demonstrated recently that slow protein diffusion can lead to large spatial gradients of cellular phospho-proteins [15]. Here we evaluate the extent to which protein diffusion limits the transfer of phosphorylation signals within the cell, and how spatial gradients are controlled by the protein kinase, the phosphatase and diffusion. The results determine the conditions under which

Abbreviations used: MAPK, mitogen-activated protein kinase; ERK, extracellular-signal-regulated kinase; MEK, MAPK/ERK kinase; MCA, Metabolic Control Analysis.

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protein diffusion has significant control over the phosphorylation of signalling proteins.

RESULTS

Phosphorylation cascade with the kinase and the phosphatase localized to two different cellular sites

The simplest system for estimating diffusion control in a protein phosphorylation cycle is one where the kinase and phosphatase are localized to different membranes (or other cellular sites) separated by a distance L (Figure 1, upper panel). The phosphorylated (p) and unphosphorylated (u) forms of a protein diffuse between the membranes. We designate by p_L and u_L the concentrations of the phosphorylated and unphosphorylated forms at the surface of the membrane containing the kinase (i.e. the site where u_L is phosphorylated); p_0 and u_0 are the corresponding concentrations at the membrane where the phosphatase is located (i.e. where p_0 is dephosphorylated). At any steady state, the diffusion fluxes of the phosphorylated and unphosphorylated forms must be equal. Provided that the diffusion coefficient, D, is the same for both forms, p and u, the corresponding spatial gradients should be equal too, i.e. $\partial p/\partial x = -\partial u/\partial x$, where x is the spatial co-ordinate (for simplicity we assume that the membranes are so large that one-dimensional diffusion can be considered). Then it follows that the sum (c) of the steady-state concentrations of the forms, p and u, is constant at any point between the membranes, for instance $p_L+u_L = p_0+u_0 = c$. Therefore we can monitor the concentrations of the phospho-protein only. These are determined by the condition of equality of the steady-state rates of the kinase, phosphatase and diffusion:

$$J = v_{kin} = D\cdot\Delta/L^2 = v_p \quad (1)$$

where J is the steady-state flux; Δ ($= p_L - p_0$) is the steady-state difference (gradient) of the phospho-protein concentrations between the membranes, and v_{kin} and v_p are the rates of the kinase and phosphatase respectively. The kinase rate, v_{kin}, is a function of the concentration of the unphosphorylated form ($u_L = c - p_L$) near the membrane containing the kinase. The phosphatase rate, v, depends on the concentration of the phosphorylated form, p_0, near the other membrane, where the phosphatase is located. The gradient of the phosphorylated form, $p_L - p_0$, equals the gradient of unphosphorylated form, $u_0 - u_L$.

The control exerted by the kinase, the phosphatase and diffusion over the phosphorylation rate (J) or the phospho-protein gradient (Δ) is quantified as the corresponding control coefficient (C_k^Y) [7,8]:

$$C_k^Y = \lim_{\delta k\to 0}\frac{\delta Y/Y}{\delta k/k} = \frac{\partial \ln Y}{\partial \ln k} \quad (2)$$

Here Y stands for J or Δ, and k is the activity (V_{max} or the observed first-order rate constant) of the kinase or phosphatase, or the diffusion coefficient.

When both the kinase and phosphatase are far from saturation by their substrates (i.e. in a quasi-linear domain), the rate expressions in eqn (1) reduce to $v_{kin} = k_{kin}\cdot(c - p_L)$ and $v_p = k_p\cdot p_0$. Here, k_{kin} and k_p are the observed first-order rate constants of the kinase and phosphatase respectively, which are expressed through the Michaelis–Menten kinetic parameters as $V_{max}/K_m = k_{cat}\cdot[E]/K_m$ (where [E] is the enzyme concentration). Within this quasi-linear kinetic domain, the explicit solution to eqn (1) reads:

$$J = c\cdot\Omega;$$

$$\Delta = [c/(D/L^2)]\cdot\Omega;$$

$$\Omega = [1/k_{kin} + 1/(D/L^2) + 1/k_p]^{-1} \quad (3)$$

Using eqns. (2) and (3), the control coefficients of the kinase (C_{kin}^J), of diffusion (C_d^J) and of the phosphatase (C_p^J) over the phosphorylation flux (J) can be calculated readily. Since the terms $1/k_{kin}$, $1/(D/L^2)$ and $1/k_p$ represent the characteristic times (τ) of the kinase, diffusion and phosphatase reactions respectively, the control coefficients can be expressed in terms of the characteristic times of the three underlying processes:

$$C_{kin}^J = \tau_{kin}/(\tau_{kin}+\tau_d+\tau_p); \quad C_d^J = \tau_d/(\tau_{kin}+\tau_d+\tau_p);$$

$$C_p^J = \tau_p/(\tau_{kin}+\tau_d+\tau_p); \quad \tau_{kin} = 1/k_{kin};$$

$$\tau_d = 1/(D/L^2); \quad \tau_p = 1/k_p \quad (4)$$

Eqn (4) clearly demonstrates that the control is distributed according to characteristic times, i.e. the slower the process, the

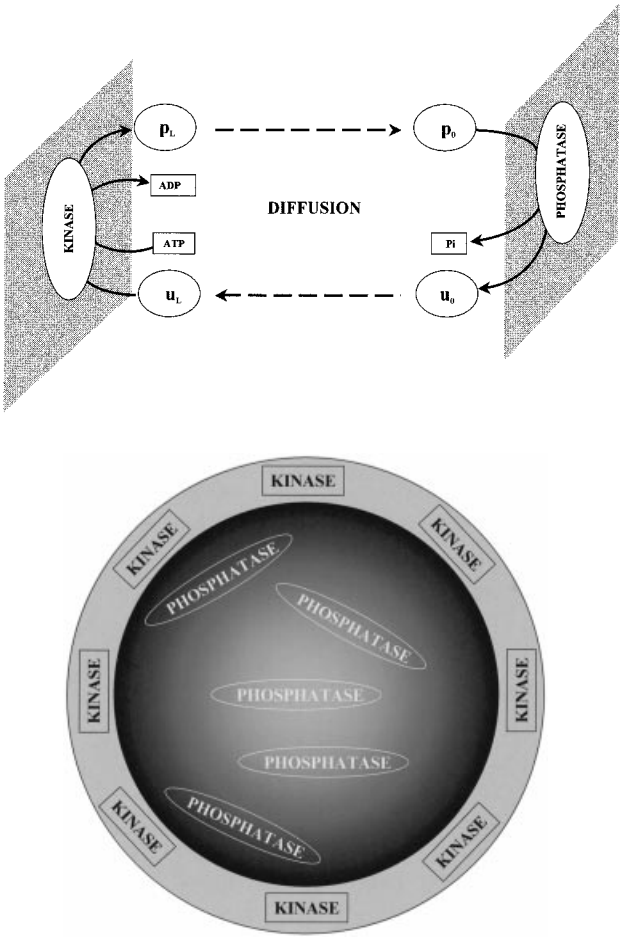

Figure 1 Schematic presentation of a phosphorylation cascade in which the kinase and phosphatase are separated spatially

Upper panel: the kinase and the phosphatase are localized to two different cellular membranes. L is the distance between the membranes; p_L and u_L are the concentrations of the phosphorylated and unphosphorylated forms of a protein at the surface of the membrane with the kinase; p_0 and u_0 are the corresponding concentrations at the membrane where the phosphatase is located. The phosphorylated form diffuses from the membrane with the kinase to the membrane with the phosphatase, and the unphosphorylated form diffuses in the opposite direction (diffusion shown by broken arrows). Lower panel: the kinase is located on the cell membrane and the phosphatase is distributed homogeneously in the cytoplasm. The phospho-protein diffuses from the plasma membrane to the cell interior. The spatial gradient of the phospho-protein is shown schematically as a gradient of grey.

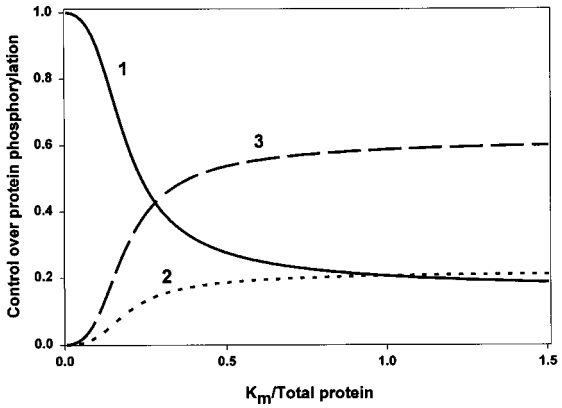

Figure 2 Dependence of the control distribution on the degree of saturation of the kinase reaction

The flux control coefficients of the kinase (curve 1), the phosphatase (curve 2) and diffusion (curve 3) are shown. The kinase and the phosphatase were assumed to follow Michaelis–Menten kinetics. The K_m value for the phosphatase was assumed to be 100 nM, which coincides with the total concentration (c) of the phosphorylated and unphosphorylated forms of the protein (c = 100 nM). The V_{max} value of the phosphatase was taken to be 100 nM/s, so that the observed first-order rate constant $k_p = V_{max}/K_m = 1\ s^{-1}$. For the kinase, the ratio $k_{kin} = V_{max}/K_m = 1\ s^{-1}$ remained unchanged while the K_m value decreased, so that the K_m/c ratio varied from 0.01 to 1.5.

higher its control coefficient. Therefore, in order to estimate how the phosphorylation flux is controlled, all we need now is to compare the observed first-order rate constants of (i) one-dimensional diffusion (D/L^2), (ii) the kinase (k_{kin}) and (iii) the phosphatase (k_p). The diffusion coefficient (D) of soluble proteins in the cytosol has been measured to be of the order of $10^{-8}\ cm^2 \cdot s^{-1}$ [16–19], although it can be considerably lower if the protein binds reversibly to immobile components of the cell [20]. The k_{kin} and k_p values have been estimated to vary from roughly 0.1 to $10\ s^{-1}$ and from 0.1 to $100\ s^{-1}$ respectively ([15] and references therein; [21,22]). When L varies from 0.1 to 10 μm, D/L^2 varies from 100 to $0.01\ s^{-1}$. Therefore eqn (4) demonstrates that, if the distance between the membranes, L, is equal to or less than 0.1 μm, diffusion does not limit protein phosphorylation flux. However, if L is several μm, the contribution of diffusion to the control can be substantial. For instance, for $L = 1$, 2 and 5 μm and $k_{kin} = k_p = 1\ s^{-1}$, the control by diffusion reaches 33, 66 and 92 % respectively.

We may usefully ask what happens if either the kinase or the phosphatase is saturated. For any given kinetics of the kinase and phosphatase, Appendix A expresses the control coefficients into so-called 'elasticities' (eqn A 3), determined by particular kinetic properties of enzymes in the phosphorylation cycle. The elasticity is the sensitivity of the reaction rate to a change in the concentration of its substrate or product, and it decreases when the enzyme approaches the saturation condition. The control exerted by an enzyme generally increases with a decrease in its elasticity; indeed, eqn (A 3) shows that the control exerted by the kinase or phosphatase increases when the enzyme becomes saturated. Accordingly, the control by diffusion decreases in this case. In order to illustrate this numerically, in our example above we leave the observed first-order rate constants (V_{max}/K_m) of the kinase and the phosphatase unchanged ($k_{kin} = k_p = 1\ s^{-1}$), while decreasing the ratio of the K_m of the kinase to the total protein concentration (c). When the distance between the membranes equals 2 μm, Figure 2 demonstrates that the control exerted by diffusion (curve 3) decreases from 0.6 to 0.1 when the K_m/c ratio decreases from 1.5 to 0.1 (i.e. when the kinase switches from a quasi-linear domain to saturation), whereas the control by the kinase increases from 0.15 to approx. 0.85 (curve 1).

For an understanding of the dynamic organization of cell signalling, knowledge of phospho-protein gradients and the control distribution over those gradients is more critical than information about the phosphorylation flux and its control. Notably, in the case of one-dimensional diffusion the flux through a phosphorylation cascade is merely proportional to the spatial gradient of a phospho-protein (eqn 1). It then follows from eqns (1) and (2) that the control coefficients of the kinase and phosphatase over the gradient (Δ) are equal to the corresponding control coefficients over the phosphorylation flux (J), but the control by diffusion over the gradient equals the control over the flux minus 1:

$$C^{\Delta}_{kin} = C^{J}_{kin}; \quad C^{\Delta}_{d} = C^{J}_{d} - 1; \quad C^{\Delta}_{p} = C^{J}_{p} \tag{5}$$

Therefore the control of diffusion over the gradient is always negative, whereas the control exerted by the kinase or phosphatase is positive, i.e. an increase in the diffusion coefficient will decrease the gradient, whereas an increase in the rate constant of the kinase or phosphatase will increase the gradient. Note that, in contrast with the control over the flux, the sum of the control coefficients over the phospho-protein gradient is zero. Indeed, equal proportional changes in the first-order rate constants of the kinase, phosphatase and diffusion do not change the concentrations of a phospho-protein, whereas the flux increases proportionally.

Because of the simplicity of the relationship given by eqn (5), Figure 2 is helpful for illustrating how the control over the phospho-protein gradient changes under saturation conditions. Curves 1 and 2 correspond to the control exerted by the kinase and the phosphatase respectively over the gradient, whereas the control exerted by diffusion can be visualized simply by shifting curve 3 into negative territory by adding -1.

When neither the kinase nor the phosphatase are saturated by the target protein, the spatial gradient of the phospho-protein (Δ) is given by eqn (3). In this case, the control coefficients can be expressed in terms of the concentrations of the phosphorylated form near the membranes and the diffusion gradient, as follows:

$$C^{\Delta}_{kin} = 1 - p_L/c; \quad C^{\Delta}_{d} = \Delta/c - 1; \quad C^{\Delta}_{p} = p_0/c \tag{6}$$

It is instructive to examine what happens when the diffusion coefficients of the phospho-protein (D_p) and of its unphosphorylated form (D_u) are different. For instance, this may occur if the phospho-protein binds reversibly to some cytosolic structures, whereas the unphosphorylated form diffuses freely. In this case, the spatial gradients of the forms p and u will no longer be identical. For the two-membrane geometry, the concentration gradients are inversely proportional to the corresponding diffusion coefficients:

$$p_L - p_0 = (D_u/D_p) \cdot (u_0 - u_L) \tag{7}$$

This demonstrates clearly that the sum of the steady-state concentrations of the phosphorylated and unphosphorylated forms is no longer independent of the location between the membrane. Instead, the 'weighted' sum, $p + (D_u/D_p) \cdot u = w$, is constant at any point.

In order to quantify the control, we now have to define two control coefficients with respect to D_p and D_u. Importantly, the consideration above for equal diffusion coefficients can be applicable if we determine the control exerted by diffusion as the sum of these two control coefficients:

$$C^{Y}_{d} = C^{Y}_{D_p} + C^{Y}_{D_u} \tag{8}$$

where Y denotes the phosphorylation flux or concentration gradient. Note that this definition of the diffusion control corresponds to equal relative modulation of either diffusion coefficient, D_p and D_u, which leaves the ratio D_u/D_p unchanged. When both the kinase and the phosphatase are in a quasi-linear kinetic domain, the explicit expression for the steady-state flux and spatial gradients of the phosphorylated (Δ_p) and un-phosphorylated (Δ_u) forms reads:

$$J = w\cdot\Omega;\quad \Delta_p = [w/(D_p/L^2)]\cdot\Omega;\quad \Delta_u = [w/(D_u/L^2)]\cdot\Omega;$$
$$\Omega = [1/k_{\mathrm{kin}}\cdot D_u/D_p + 1/(D_p/L^2) + 1/k_{\mathrm{p}}]^{-1} \qquad (9)$$

From eqns (8) and (9), it then follows the control exerted by the kinase, diffusion and the phosphatase is determined by eqn (4) after substitution of the diffusion coefficient D by D_p in τ_{d} and multiplication of τ_{kin} by D_u/D_p.

Phosphorylation cascade with a membrane kinase and a cytosolic phosphatase

When a protein is phosphorylated by a kinase localized to the cell membrane and dephosphorylated by a phosphatase located homogeneously in the cytosol, the spatial distribution of the phospho-protein is determined by the diffusion equation, which takes into account the consumption of the protein in the phosphatase reaction (see Appendix B). The boundary conditions equate the protein phosphorylation rate at the cell membrane to the rate of protein diffusion into the cell interior, and diffusion flux equals zero at the centre of the cell. In contrast with the situation described above where the kinase and phosphatase are localized to different membranes, the analytical machinery of MCA cannot be applied to calculate the control coefficients from the elasticities. Indeed, the connectivity theorems of MCA require the presence of a finite number of explicit intermediates [23,24], which is not the case when the reaction rates change with the spatial co-ordinate. However, the control coefficients can be determined either by direct differentiation of the solution to reaction–diffusion equations (Appendix B), or by taking the appropriate variations of these equations [25]. Importantly, the summation theorems of MCA continue to apply. The sum of the control coefficients of the kinase, the phosphatase and diffusion over the phosphorylation rate equals unity, whereas the total control over the phospho-protein gradient (defined as the phospho-protein concentration difference between the plasma membrane and cell centre) equals zero.

For a spherical cell of radius L and with both the kinase and the phosphatase far from saturation, the control pattern and the phospho-protein gradient are determined by the observed first-order rate constants of the kinase (k_{kin}), by the radial diffusion ($3D/L^2$) and by a dimensionless parameter (α) which depends on the observed first-order rate constants of the phosphatase (k_{p}) and diffusion [$\alpha^2 = k_{\mathrm{p}}/(D/L^2)$] (see Appendix B):

$$C^J_{\mathrm{kin}} = \frac{(3D/L^2)[e^{2\alpha}(\alpha-1)+\alpha+1]}{(3D/L^2)[e^{2\alpha}(\alpha-1)+\alpha+1]+k_{\mathrm{kin}}(e^{2\alpha}-1)},$$
$$\alpha = L\cdot\sqrt{k_p/D};\quad C^J_{\mathrm{p}} + C^J_{\mathrm{d}} = 1 - C^J_{\mathrm{kin}} \qquad (10)$$

For a cell radius (L) of 10 μm and $D = 10^{-8}$ cm$^2\cdot$s^{-1}, the observed diffusion rate constant ($3D/L^2$) is 0.03 s^{-1}. When the k_{p} values are greater than 0.1 s^{-1} (which is true for most phosphorylation cascades), the parameter α is greater than 3.2, and the exponential terms in eqn (10) become predominant. Then eqn (10) shows that the protein phosphorylation rate is controlled mostly by the phosphatase and diffusion, unless the kinase activity is significantly lower than the phosphatase activity. Indeed, $C^J_{\mathrm{p}} + C^J_{\mathrm{d}} \geqslant C^J_{\mathrm{kin}}$, if $k_{\mathrm{kin}} \geqslant (3D/L^2)(\alpha-1)$. For instance, if $k_{\mathrm{p}} = 0.1$ or 100 s^{-1}, the control of the phosphorylation flux will reside mainly on the phosphatase and diffusion, provided that k_{kin} is greater than 0.06 or 3 s^{-1} respectively. This is illustrated by Figure 3, which shows how the control exerted over the phosphorylation flux by the kinase (curve 1), the phosphatase (curve 2) and diffusion (curve 3) depends on the phosphatase (Figure 3A) and kinase (Figure 3B) activities. One of the striking features of this control pattern is a significant control contribution of protein diffusion. With an increase in the kinase activity, the extent to which the phosphorylation flux is limited by diffusion increases monotonically, whereas, with an increase in the phosphatase activity, the diffusion limitation increases only at low k_{p} values, and then decreases slowly (curve 3). Notably, when the kinase and phosphatase activities are localized to different membranes, the diffusion limitation increases monotonically with an increase in both k_{kin} and k_{p}.

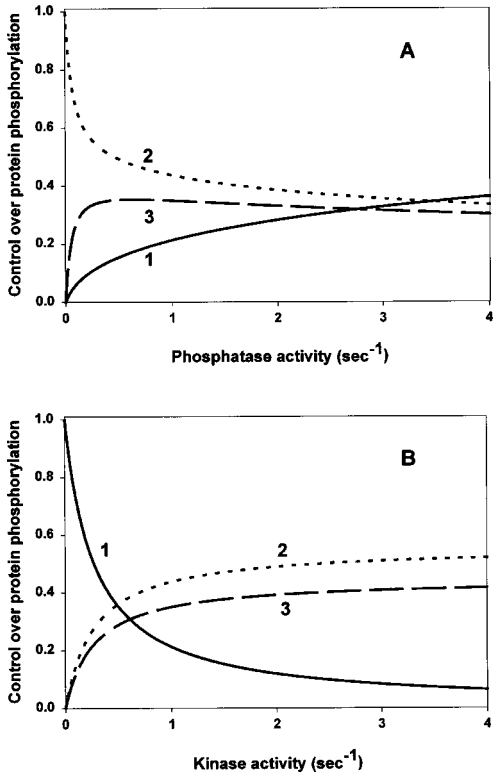

Figure 3 Control distribution over protein phosphorylation flux

The dependence of the control coefficients of the kinase (curve 1), the phosphatase (curve 2) and diffusion (curve 3) on the phosphatase (**A**) and kinase (**B**) activities is shown. The parameter values are: $D/L^2 = 0.01$ s^{-1}, (**A**) $k_{\mathrm{kin}} = 1$ s^{-1}, (**B**) $k_{\mathrm{p}} = 1$ s^{-1}.

For a spherical cell, the control over the spatial gradient of a phospho-protein can be determined in a similar manner (see Appendix B). When the phosphatase activity is distributed over the cytosol, the control by diffusion over the protein phosphorylation flux is no longer proportional to the phospho-protein gradient, in contrast with the case when the kinase and the phosphatase are located on two different membranes. Figure 4 illustrates how the control over the phospho-protein gradient depends on the observed first-order rate constant of the phosphatase (Figure 4A) and diffusion (Figure 4B). The control by diffusion is negative (curve 3) and that by the kinase is positive (curve 1), similar to the situation where the kinase and

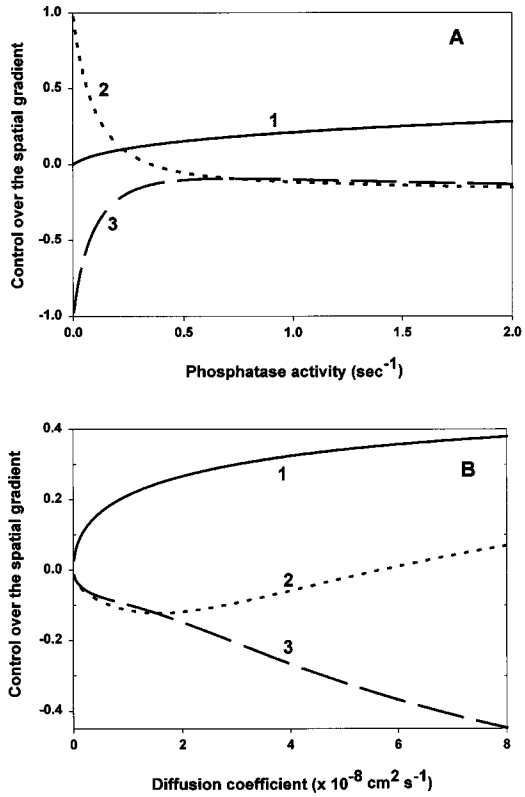

Figure 4 Control over the phospho-protein gradient

The dependence of the control coefficients of the kinase (curve 1), the phosphatase (curve 2) and diffusion (curve 3) on phosphatase activity (**A**) and the diffusion coefficient (**B**) is shown. The parameter values are: $k_{kin} = 1\ s^{-1}$, (**A**) $D/L^2 = 0.01\ s^{-1}$, (**B**) $k_p = 1\ s^{-1}$.

phosphatase activities were localized to different membranes (see above). An increase in the diffusion coefficient decreases the spatial gradient for any geometry of the distribution of the kinases and phosphatases. However, in sharp contrast with the two-membrane geometry, the control exerted by the phosphatase over the phospho-protein gradient can be negative (curve 2), i.e. the gradient can decrease with an increase in k_p. Interestingly, when the phosphatase activity is low and the spatial gradient of the phospho-protein is not large, the diffusion control over the phosphorylation flux can be substantial.

The ratio of the concentration of a phospho-protein at the plasma membrane and its concentration at the cell centre ($\mu = p_L/p_0$) can be considered as a quantitative indicator of the dynamic compartmentation of phospho-protein signalling. When the cytosolic phosphatase is not saturated by the target protein, this ratio does not depend on the kinase activity and is a function of the dimensionless parameter (α) only (Appendix B):

$$\mu = (p_L/p_0) = (e^{2\alpha} - 1)/(2\alpha \cdot e^{\alpha}) \quad (11)$$

As a consequence, the control over the ratio p_L/p_0 by the kinase equals zero, whereas the control coefficients of the phosphatase and diffusion have the same absolute values, but different signs:

$$C^{\mu}_{k_p} = \frac{(\alpha-1)\,e^{2\alpha} + \alpha + 1}{2(e^{2\alpha} - 1)};$$

$$C^{\mu}_{d} = -\frac{(\alpha-1)\,e^{2\alpha} + \alpha + 1}{2(e^{2\alpha} - 1)} \quad (12)$$

The parameter α was estimated above to be greater than 3.2. Therefore in eqn (12) the exponential terms are predominant and the control coefficients of the phosphatase and diffusion tend to $(\alpha-1)/2$ and $-(\alpha-1)/2$ respectively.

DISCUSSION

Signal transduction within the live cell may involve substantial cellular gradients of phospho-proteins, given the measured rates of spatially separated protein kinases, phosphatases and diffusion [15]. In the present paper we have derived relationships between the rate constants of these three processes and the control exerted by these processes over the phosphorylation flux and the spatial gradient of a phospho-protein within a cell. These relationships enable us to calculate the extent to which a kinase, a phosphatase or diffusion limits the flux or concentration gradient if we know the rate constants and cellular dimensions. If the ratio of the diffusion coefficient of a phospho-protein to the diffusion distance squared (D/L^2) becomes low relative to the rate constants of the kinase and the phosphatase, then protein phosphorylation becomes rate-limited by diffusion. For a cell in which the protein kinase is localized to the plasma membrane and the phosphatase is distributed throughout the cytosol, eqn (11) provides a simple but powerful criterion of the existence of a large phospho-protein gradient, i.e. $\sqrt{k_p/(D/L^2)} \gg 1$, where $k_p = V_{max}/K_m$ is the observed first-order rate constant of the phosphatase.

In some cases, having large spatial gradients of phospho-proteins can be an advantage for cell signalling [26,27]. For example, the active form of a cytosolic protein phosphorylated at the membrane may be effectively restricted to a narrow domain below the membrane. If this protein is an enzyme acting on membrane phospholipids (such as phospholipase C-γ or phosphoinositide 3-kinase), this spatial distribution has an additional function to keep the active enzyme in a close proximity to its substrate. However, if the targets are in the cytoplasm, the diffusion limitation on the protein phosphorylation flux may be a disadvantage. We may ask how the control contribution by diffusion could be decreased or eliminated within the cell. Scaffolding of multicomponent kinase/phosphatase cascades may be a major mechanism. For instance, a cytosolic phosphatase can bind to an activated membrane receptor kinase, so that the receptor functions as a scaffold for both activities. The proximity of the kinase and the phosphatase decreases or eliminates diffusion limitations. Emerging evidence indicates that specific scaffold proteins organize and co-ordinate the function of multiple signal transduction cascades [28–30].

Diffusion limitation of fluxes has not previously been analysed by MCA; indeed, the existence of spatial distributions of metabolite concentrations has been thought to prevent the meaningful application of MCA. We demonstrate here that diffusion limitation can be analysed and quantified within the MCA framework, in much the same way as other processes. In cellular metabolic pathways in which different enzymes are not separated spatially into different compartments, the flux of metabolites is not limited by diffusion [12]. However, there are cases when the enzymes that produce and consume a metabolite are separated spatially, e.g. ATP synthesis in mitochondria and its consumption in the cytoplasm. The approach we have used here might be adapted to the analysis of limitations of cellular ATP turnover by ATP/ADP diffusion in the cytoplasm [31,32], and to other diffusion problems.

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APPENDIX A

The control coefficients are determined by the geometry (structure) of a pathway and the kinetic properties of molecular processes (reactions) described by the elasticity coefficients in MCA. The elasticity (ϵ) is the sensitivity of the reaction rate (v) to a change in the concentration (S) of its substrate or product, and is defined as:

$$\epsilon_S^v = \partial(\ln v)/\partial(\ln S) \quad \text{(A 1)}$$

The elasticity coefficient depends on the kinetic properties of a particular process only, and it is equal to zero unless S affects v directly. For the phosphorylation pathway under consideration, v stands for the rate of the kinase, the phosphatase or diffusion, and S stands for p_L or p_0. The difference between the elasticities and control coefficients is that the concentrations of all the components except x are held constant to measure ϵ, but they are allowed to reach a new steady state when the control coefficients are estimated. The control coefficients can be expressed in terms of the elasticity coefficients using the so-called summation and connectivity theorems of MCA [7,8], which for the phosphorylation pathway under consideration read:

$$C^J_{\rm kin} + C^J_{\rm d} + C^J_{\rm p} = 1; \quad C^J_{\rm kin}\cdot\epsilon^{\rm kin}_{p_L} + C^J_{\rm d}\cdot\epsilon^{\rm d}_{p_L} = 0;$$
$$C^J_{\rm d}\cdot\epsilon^{d}_{p_0} + C^J_{\rm p}\cdot\epsilon^{p}_{p_0} = 0 \quad \text{(A 2)}$$

where the indexes kin, p and d denote the kinase, the phosphatase and diffusion respectively. The solution to the linear equation system (A2) can be found readily:

$$C^J_{\rm kin} = -\epsilon^{\rm d}_{p_L}\cdot\epsilon^{\rm p}_{p_0}\cdot\theta; \quad C^J_{\rm d} = \epsilon^{\rm kin}_{p_L}\cdot\epsilon^{\rm p}_{p_0}\cdot\theta;$$
$$C^J_{\rm p} = -\epsilon^{\rm kin}_{p_L}\cdot\epsilon^{\rm d}_{p_0}\cdot\theta; \quad \theta = (\epsilon^{\rm kin}_{p_L}\cdot\epsilon^{\rm p}_{p_0} - \epsilon^{\rm d}_{p_L}\cdot\epsilon^{\rm p}_{p_0} - \epsilon^{\rm kin}_{p_L}\cdot\epsilon^{\rm d}_{p_0})^{-1} \quad \text{(A 3)}$$

APPENDIX B

For a spherical cell, the following equation describes the radial diffusion of the phosphorylated form (p) from the cell membrane (where p is produced) into the cytosol (where p is dephosphorylated by the uniformly distributed phosphatase):

$$\frac{\partial p}{\partial t} = \frac{D}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial p}{\partial r}\right) - v_{\rm p} \quad \text{(B 1)}$$

At the cell membrane ($r = L$), the rate of protein phosphorylation by the kinase is equal to the rate of phospho-protein diffusion into the cell interior (all the rates are based on the unit of the cell volume); at the cell centre ($r = 0$) there should be no diffusion flux:

$$v_{\rm kin} = 3\frac{D}{L}\frac{\partial r}{\partial r}\bigg|_{r-L}; \quad \frac{\partial p}{\partial r}\bigg|_{r=0} = 0 \quad \text{(B 2)}$$

In eqns. (B1) and (B2), $p(r)$ is the concentration of the phosphorylated form of the protein at distance r from the centre of the cell, and L is the cell radius. The phosphatase rate $v_{\rm p}$ depends on $p(r)$, and the kinase rate $v_{\rm kin}$ depends on the concentration of the unphosphorylated form near the surface of the cell membrane, i.e. $u_L = c - p_L$, where p_L and u_L stand for $p(L)$ and $u(L)$, and c is the total concentration of the phosphorylated and dephosphorylated forms, which is constant throughout the cell (for simplicity, the same diffusion coefficient is assumed for both forms, p and u). Here we will consider the steady-state solution to eqn (B1), determined by equating $\partial p/\partial t = 0$.

The control coefficients are defined according to eqn (2) of the main text as the log-to-log derivatives of the steady-state reaction rates or concentrations (designated Y in eqn 2) with respect to the catalytic activities and the diffusion coefficient (designated k). First, we calculate the non-normalized derivatives of the concentrations $p(r)$, i.e. the sensitivities, $s_k(r) = \partial p/\partial k$. These satisfy a linear inhomogeneous partial differential equation obtained by differentiation of eqns (B1) with respect to the parameter k ('variation' equation) [24,25]:

$$\frac{D}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial s_k}{\partial r}\right) - \frac{\partial v_{\rm p}}{\partial p}\cdot s_k + h = 0;$$
$$h = \frac{\partial D}{\partial k}\cdot\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial p}{\partial r}\right) - \frac{\partial v_{\rm p}}{\partial k} \quad \text{(B 3)}$$

with the boundary conditions obtained by differentiation of eqns (B2):

$$\frac{\partial v_{\rm kin}}{\partial k} + \frac{\partial v_{\rm kin}}{\partial p_L}\cdot s_k(L) = 3\frac{D}{L}\frac{\partial s_k}{\partial r}\bigg|_{r-l} + 3\frac{\partial(D/L)}{\partial k}\cdot\frac{\partial p}{\partial r}\bigg|_{r-L}; \quad \frac{\partial s_k}{\partial r}\bigg|_{r=0} = 0 \quad \text{(B 4)}$$

When the concentration profile, $p(r)$, is found, the linearity of the equation for the sensitivity allows us to calculate its solution (which can be written, for example, in terms of a Green's function [25]).

When the sensitivities, $s_k(r)$, are obtained, the control coefficients are calculated readily. For instance, the control over the steady-state flux through the kinase (J) is expressed as follows:

$$C^J_{\rm kin} = 1 + s_{k_{\rm kin}}(L)\cdot\frac{k_{\rm kin}}{p_L}\cdot\epsilon^{\rm kin}_{p_L}; \quad C^J_{\rm d} = s_{\rm d}(L)\cdot\frac{D}{p_L}\cdot\epsilon^{\rm kin}_{p_L};$$
$$C^J_{\rm p} = s_{k_{\rm p}}(L)\cdot\frac{k_{\rm p}}{p_L}\cdot\epsilon^{\rm kin}_{p_L}; \quad \epsilon^{\rm kin}_{p_L} = \frac{\partial\ln v_{\rm kin}}{\partial\ln p_L} \quad \text{(B 5)}$$

where the indexes kin, p and d denote the kinase, phosphatase and diffusion respectively, $k_{\rm kin}$ and $k_{\rm p}$ stand for the catalytic activities ($V_{\rm max}$ or first-order rate constant) of the kinase and the phosphatase respectively, and ϵ is the elasticity coefficient. The control coefficients over a flux (J) or the concentration [$p(r)$] satisfy the following summation theorems:

$$C^J_{\rm kin} + C^J_{\rm d} + C^J_{\rm p} = 1; \quad C^p_{\rm kin} + C^p_{\rm d} + C^p_{\rm p} = 0 \quad \text{(B 6)}$$

When the phosphatase is not saturated by the target protein, i.e. $v_{\rm p} = k_{\rm p}\cdot p$, the steady-state solution to eqn (B1) reads:

$$p(r) = \text{const.}\cdot(e^{\lambda r} - e^{-\lambda r})/r; \quad \lambda = \sqrt{k_p/D} \quad \text{(B 7)}$$

The ratio $\mu = p_L/p_0$ (see eqn 11 of the main text) is obtained by substituting $r = L$ and $r = 0$ respectively into eqn (B7). The constant factor in eqn (B7) is determined by the kinase activity. If the kinase is far from saturation, $v_{\rm kin} = k_{\rm kin}\cdot(c - p_L)$, then the constant factor in $p(r)$ is given by:

$$\text{const.} = \frac{ck_{\rm kin}L\cdot e^{\alpha}}{(3D/L^2)[e^{2\alpha}(\alpha-1)+\alpha+1] + k_{\rm kin}(e^{2\alpha}-1)};$$
$$\alpha = L\cdot\sqrt{k_{\rm p}/D} \quad \text{(B 8)}$$

Substituting eqn (B 8) into eqn (B 7), we obtain p_L and p_0. Then the steady-state flux (J) through the kinase and the phospho-protein gradient ($\Delta = p_L - p_0$) are expressed as:

$$J = \frac{ck_{\mathrm{kin}}(3D/L^2)[e^{2\alpha}(\alpha-1)+\alpha+1]}{(3D/L^2)[e^{2\alpha}(\alpha-1)+\alpha+1]+k_{\mathrm{kin}}(e^{2\alpha}-1)} \quad \text{(B 9)}$$

$$\Delta = \frac{ck_{\mathrm{kin}}(e^{2\alpha}-2\alpha e^{\alpha}-1)}{(3D/L^2)[e^{2\alpha}(\alpha-1)+\alpha+1]+k_{\mathrm{kin}}-(e^{2\alpha}-1)} \quad \text{(B 10)}$$

The control coefficients of the kinase, the phosphatase and diffusion over the protein phosphorylation rate (J) or the phospho-protein gradient (Δ) are calculated by direct differentiation of eqns. (B9) and (B10) with respect to k_{kin}, k_{p} and D respectively. In this manner, eqn (10) of the main text is derived, e.g. $C^{J}_{\mathrm{kin}} = \partial(\ln J)/\partial(\ln k_{\mathrm{kin}})$. Eqn (12) of the main text is obtained by differentiation of $\mu = p_L/p_0$ with respect to k_{p} and D.

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