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Regulation of kinase activity by diffusion and feedback

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ABSTRACT

In living cells proteins motilities regulate the spatiotemporal dynamics of molecular pathways. We consider here a reaction–diffusion model of mutual kinase–receptor activation showing that the strength of positive feedback is controlled by the kinase diffusion coefficient. For high diffusion, the activated kinase molecules quickly leave the vicinity of the cell membrane and cannot efficiently activate the receptors. As a result, in a broad range of parameters, the cell can be activated only if the kinase diffusion coefficient is sufficiently small. Our simple model shows that change in the motility of substrates may dramatically influence the cell responses.

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1. Introduction

Regulatory network process cellular signals in time and space enabling cell self-organization (see Kholodenko, 2006; Karsenti, 2008 for reviews). The temporal dynamics is coupled with spatial gradients of concentrations or activity. For example, kinase cascades can emerge from receptors and transmit signals from the cell membrane to the nucleus. In this case the gradient of active kinase activity develops since phosphorylation and dephosphorylation proceed at different cellular locations, respectively, cell membrane and cell volume. Due to the estimations of Brown and Kholodenko (1999), basing on measured values of protein diffusion coefficients and phosphatase activities, gradients of kinase activity are potentially very large. In a simple system in which kinase molecules are phosphorylated at the cell membrane and dephosphorylated by a phosphatase molecules located homogeneously in the cell cytosol (analyzed by Brown and Kholodenko, 1999) small diffusion implies high gradient and low kinase activity in the cell center. The problem of receptor–kinase interaction has been also studied in the context of diffusion with obstacles in the stochastic numerical simulations of bacterial chemotaxis (Lipkow et al., 2005). One of the conclusions of Lipkow et al. (2005) is that crowding results in a fall of the apparent diffusion coefficient and at the anterior end, where CheY is phosphorylated, the local concentration of CheYp increases and therefore accelerates the response of the anterior close motor. At

the other, posterior, end of the cell, the local CheYp concentration is reduced by the need to diffuse through the obstacles and the responses of motors in this region is consequently delayed.

Here we consider a similar model to the one analyzed by Brown and Kholodenko (1999), but assume the mutual receptor–kinase activation. Membrane receptors can bind extracellular ligands, that leads to cascade of molecular processes inside the cell and formation of the active receptor complex. In many cases, receptor activation requires phosphorylation. Almost all G-protein coupled receptors (GPCRs) are regulated by phosphorylation, see Tobin (2008) for review. Engagement of immunoreceptors (TCR, BCR, FcR) leads to activation of different members of the Src kinase family, which includes Lck (for T-cell, Housden et al., 2003), Fyn and Lyn (for B and mast cells, Gauld and Cambier, 2004). Src kinases then phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) contained within the immunoreceptors themselves or in receptor-associated molecules, see Abram and Lowell (2007) for review. This may lead to positive feedback, in which active receptors send signal to kinase and in turn are activated by the same kinase species or by one of the downstream kinases. In this study we consider the simplest situation, in which receptors are activated by the same kinase species they activate.

We will show, that in the case of mutual receptor–kinase activation, in a broad range of parameters controlling the process, the cell becomes activated only if the kinase diffusion is sufficiently small. For large diffusion, the activated kinase molecules quickly leave vicinity of cell membrane, and the positive feedback coupling kinases with receptors becomes inefficient.

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2. Model formulation

As said, we will assume that membrane receptors bind extracellular ligands, that leads to a cascade of processes and receptor activation. At constant extracellular cytokine concentration, a steady state uniform surface concentration of ligand-bound receptors $P = const$ is established. We will consider two cases. Firstly, following Brown and Kholodenko (1999) we will assume that all ligand-bound receptors are active. Secondly, we will assume that the limiting step in the formation of the active receptor complex is its phosphorylation by the kinase. In turn, active receptors may activate kinase molecules, that defines the positive feedback in the regulation process. The activated kinase may freely diffuse over entire cell volume, where they are inactivated by uniformly distributed phosphatases.

The cell will be modeled geometrically as a ball $B(0, r_0)$ of radius r_0 , centered at the origin of the coordinate system. We restrict to the spherically symmetric case and we will use the following notation:

- $K(t, r)$ the concentration of the active kinase
- $Q = const$ the total concentration of the kinase
- $R(t)$ the surface concentration of the active receptors
- $P = const$ the total surface concentration of the ligand bound receptors (active and inactive)
- $\Phi_K(t)$ the flux of the active kinase

The active kinase concentration satisfies

$$\frac{\partial K}{\partial t} = d_1 \nabla^2 K - b_1 K, \tag{1}$$

where $b_1 > 0$ is the kinase dephosphorylation rate due to the action of uniformly distributed phosphatases. The flux of the active kinase results from its phosphorylation by the surface receptors implying the Robin type boundary condition,

$$\Phi_K = a_1 R(Q - K_b) = d_1 \mathbf{n}(\nabla K)_b, \tag{2}$$

where \mathbf{n} is a unit vector normal to cell surface and subscript b denotes the boundary value for ($r = r_0$).

As already mentioned, regarding the receptors activation we will consider two different cases:

- (1) Case without feedback i.e. when activity of the receptors is independent of intracellular processes, but is controlled by binding and dissociation of some extracellular ligand (present at the constant concentration). In such a case we may assume that all the bound receptors are persistently active, i.e. $R(t) = const = P$.
- (2) Case with feedback, in which we assume that the limiting step in the receptor activation is its phosphorylation by the kinase, that defines the positive feedback in receptor-kinase activation,

$$\frac{dR}{dt} = a_2 K_b (P - R) - b_2 R. \tag{3}$$

In the further consideration we will assume that all the reaction rate coefficients, a_1, a_2, b_1, b_2 , and diffusion constant c_1 are positive. In the non-dimensional units $\tau = tb_1, \rho = r/r_0$, Eq. (1) reads

$$\frac{\partial K}{\partial \tau} = d \nabla^2 K^* - K^*, \tag{4}$$

where $d = d_1/(b_1 r_0^2)$ plays the role of the non-dimensional diffusion coefficient and $K^* = K/Q$. We may thus rewrite Eqs. (2) and (3) as

$$aR^*(1 - K_b^*) = d\mathbf{n}(\nabla K^*)_b, \tag{5}$$

$$\frac{dR^*}{dt} = qK_b^*(P^* - R^*) - bR^*, \tag{6}$$

where $a = a_1 P/b_1, R^* = R/(r_0 Q), q = a_2 Q/b_1, b = b_2/b_1$ and $P^* = P/(r_0 Q)$. From now on, for the sake of simplicity all the asterisks will be omitted. Let us notice that $\rho \in [0, 1], K \in [0, 1], R \in [0, P]$ and a, b, d, q and P are real and non-negative.

3. Results

3.1. Limit of infinite diffusion $d \rightarrow \infty, K = K(\tau), R = R(\tau)$

For the infinite diffusion, the active kinase concentration is uniform, and the system of Eqs. (4)–(6) is equivalent to the system of two ordinary equations, for $K(\tau), R(\tau)$,

$$\frac{dK}{d\tau} = 3aR(\tau)(1 - K(\tau)) - K(\tau), \tag{7}$$

$$\frac{dR}{d\tau} = qK(\tau)(P - R(\tau)) - bR(\tau). \tag{8}$$

Formally, we can obtain the above system by integrating Eq. (4) over the ball $B(0, 1)$ and using the Gauss theorem. Let us note that the compact region $D := [0, 1] \times [0, P]$ is invariant with respect to the flow generated by the above system. That is to say, if $\{K(0), R(0)\} \in D$, then for arbitrary $\tau \geq 0, \{K(\tau), R(\tau)\} \in D$. System (7)–(8) has two steady states: $\{K_1, R_1\} = \{0, 0\}$ and $\{K_2, R_2\}$, where

$$K_2 = \frac{3qaP - b}{q(3aP + 1)}, R_2 = \frac{3qaP - b}{3a(b + q)}. \tag{9}$$

For $b < 3qaP$ the steady state point $\{K_2, R_2\}$ is stable, while the point $\{K_1, R_1\}$ is unstable, for $b \geq 3qaP, K_2 < 0, R_2 < 0$ and the point $\{K_2, R_2\}$ is unstable, while the $\{K_1, R_1\}$ is stable. In other words, restricting to subdomain D , the system has one stable steady state $\{0, 0\}$, for $b \geq 3qaP$, or $\{K_2, R_2\}$ for $b < 3qaP$.

In the case without the feedback (i.e. when $R(\tau) = P$), Eq. (7) can be solved analytically,

$$K(\tau) = \left(K(0) - \frac{3aP}{3aP + 1} \right) \exp[(1 + 3aP)\tau] + \frac{3aP}{3aP + 1} \tag{10}$$

and has unique stable state $K_3 = 3aP/(1 + 3aP)$.

3.2. Finite diffusion—steady state analysis

3.2.1. Case without feedback

In spherical coordinates Eq. (4) reads

$$\frac{\partial K}{\partial \tau} = d \frac{1}{\rho^2} \frac{\partial}{\partial \rho} \left(\rho^2 \frac{\partial K}{\partial \rho} \right) \tag{11}$$

and has unique steady state solution

$$K(\rho) = \frac{K_c(e^{\alpha \rho} - e^{-\alpha \rho})}{2\alpha}, \tag{12}$$

where $\alpha = d^{-1/2}$ and $K_c = K(0)$. Using Eqs. (5) (with $R = P$) and (12) we may calculate boundary value $K_b = K(1)$

$$K_b = \frac{aP\alpha^2(e^{2\alpha} - 1)}{(1 + \alpha - a\alpha^2P + e^{2\alpha}(\alpha + a\alpha^2P - 1))} \tag{13}$$

and then

$$K_c = 2K_b\alpha/(e^\alpha - e^{-\alpha}) = \frac{2aP\alpha^3 e^\alpha}{(1 + \alpha - a\alpha^2P + e^{2\alpha}(\alpha + a\alpha^2P - 1))}. \tag{14}$$

In the limit of infinite diffusion $\alpha \rightarrow 0$, the active kinase distribution is uniform, $K(\rho) \equiv K_c$, with $K_c = K_3$. The total amount

of active kinase K_{tot} is

$$K_{tot} = 4\pi \int_0^1 \varrho^2 K(\varrho) d\varrho = 4\pi K_c \frac{\alpha \cosh(\alpha) - \sinh(\alpha)}{\alpha^3}. \quad (15)$$

Differentiating K_b , K_c and K_{tot} several times with respect to α , one can show that for $a > 0$ and $P > 0$:

- (1) K_b is a monotonically increasing function of $\alpha = d^{-1/2}$ and
- (2) K_c and K_{tot} are monotonically decreasing functions of α .

Large diffusion enhances the flux of the active kinase from the cell membrane towards the cell center. Thus, as one could expect, both K_c and K_{tot} are growing functions of diffusion coefficient. Simultaneously, the active kinase concentration close to the boundary decreases with increasing diffusion, Fig. 1.

3.2.2. Case with feedback

In the case with feedback the spatial profile of $K(\varrho)$ is the same as in the case without feedback; Eqs. (5) and (6) give us two values of K_b (or $K_c = K_b * 2\alpha / (e^\alpha - e^{-\alpha})$) corresponding to one stable and one unstable steady state solution

$$K_{c1} = 0, \quad K_{c2} = \frac{2\alpha e^\alpha (be^{2\alpha}(1-\alpha) - b(\alpha+1) + qaP\alpha^2(e^{2\alpha}-1))}{q(e^{2\alpha}-1)(1+\alpha - a\alpha^2P + e^{2\alpha}(\alpha + a\alpha^2P - 1))}. \quad (16)$$

The stable solution $K(\varrho)$ is given by

$$K(\varrho) = \frac{K_c(e^{\alpha\varrho} - e^{-\alpha\varrho})}{2\varrho\alpha}, \quad (17)$$

where $K_c = \max(K_{c1}, K_{c2})$, i.e. for $K_{c2} > 0$ the stable solution is positive, while for $K_{c2} < 0$, $K(\varrho) \equiv 0$. The global stability (with respect to a perturbation without spherical symmetry) of $K(\varrho)$ solution is proved in Appendix A.

Let us note, that in the limit of the infinite diffusion coefficient $d \rightarrow \infty$, i.e. $\alpha \rightarrow 0$, $K(\varrho) \equiv K_c = K_2$ and thus one obtains the same solution as given in Eq. (9). In the opposite limit $d \rightarrow 0$ (i.e. $\alpha \rightarrow \infty$), $K_b = 1$, $K_c = 0$, $K_{tot} = 0$. In further analysis we set $q = a = P = 1$, and consider the steady state kinase activity profiles with respect to two non-dimensional parameters: $\alpha = d^{-1/2}$ and b (receptor inactivation constant).

In Fig. 2 we analyze the dependence of the stable steady state $K(\varrho)$ on the diffusion parameter $\alpha = d^{-1/2}$. With respect to the

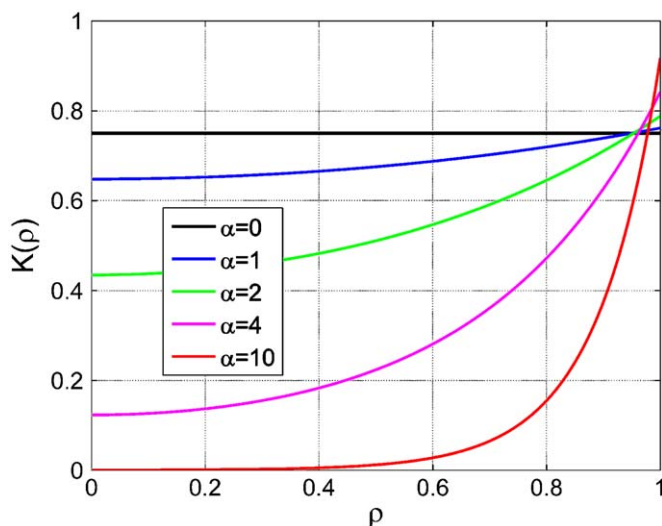


Fig. 1. The case without feedback. Profiles of active kinase concentration $K(\rho)$ for different values of $\alpha = d^{-1/2}$. For all plots the remaining parameters are fixed: $a = P = 1$.

receptor dephosphorylation coefficient b , we may distinguish three cases, shown in Panels A–C. For small b (Panel A), the dependence of active kinase concentration profiles $K(\varrho)$ on α is similar as in the case without feedback; the larger is α , the steeper is the active kinase profile, with higher value at the boundary and the lower value in the cell center. Qualitatively different is the case shown in Panel B for larger values of b . For $b = 2.5$, the active kinase concentration is higher across the whole cell for some finite diffusion ($\alpha = 2$) than for the infinite diffusion ($\alpha = 0$). In the case of large dephosphorylation parameter (Panel C, $b = 4$) for infinite, or large diffusion, $K(\varrho) \equiv 0$, while for smaller diffusion $K(\varrho) > 0$. This somehow surprising effect is due to the fact that the strength of positive feedback is controlled by the diffusion. For small diffusion activated kinase remain longer in the vicinity of the membrane and may activate the receptors more effectively.

In Figs. 3A and B we analyze $K_b(\alpha)$ and $K_c(\alpha)$ for four different values of b . As can be expected, K_b is a growing function of α . For small b , K_c decreases with growing α (as in the case without feedback); however, for larger b , $K_c(\alpha)$ has a maximum for some $\alpha_m(b) > 0$. The existence of such an “optimal” α_m is due to interplay of two counter-effects:

- (1) large diffusion (small α) speeds translocation of active kinase, so they have a larger chance to remain phosphorylated until they reach the cell center
- (2) simultaneously large diffusion attenuates the positive feedback coupling kinases with receptors.

In contrast to the case without feedback, the total amount of active kinase $K_{tot}(\alpha)$ for large b has maximum for some $\alpha'_m(b) > \alpha_m(b) > 0$. Let us note also, that both $\alpha'_m(b)$ and $\alpha_m(b)$ are growing functions of b , diverging logarithmically to infinity with b .

As shown in Fig. 4 there are unbounded parameter domains D_c and D_{tot} in (α, b) plane for which, respectively, $\Delta_c := K_c(\alpha, b) - K_c(0, b) > 0$ and $\Delta_{tot} := K_{tot}(\alpha, b) - K_{tot}(0, b) > 0$. Since for finite diffusion $K_b(\alpha) > K_c(\alpha)$: $D_c \subset D_{tot}$. In the case of the infinite diffusion ($\alpha = 0$), the positive solutions $K(\rho, \alpha, b) > 0$ are restricted to domain $b < 3$. In the case of finite diffusion, for arbitrarily large b there exists such $\alpha(b)$ that $K(\rho, \alpha, b) > 0$.

4. Discussion

Dynamics of molecular pathways is determined by both, chemical reaction rules and localization of substrates that in turn is governed by diffusion or transport. We considered here a simple theoretical model of mutual receptor–kinase activation in which the kinase molecules are phosphorylated by the receptors at the cell membrane and may freely diffuse in the cell volume, where they are dephosphorylated with time- and space-independent dephosphorylation rate. The positive feedback, considered in the model, arises since activated kinase may in turn activate receptors.

In the case without feedback, active kinase concentration in the cell center and the total amount of active kinase are monotonically growing functions of diffusion coefficient. However, the presence of the positive feedback causes that the concentration of the active kinase in the cell volume is a non-trivial function of the diffusion coefficient. The active kinase concentration in the cell center depends on the two opposite, diffusion controlled effects: the kinase activity profile along the cell radius is flatter for large diffusion and the strength of positive feedback controlling active kinase concentration at the boundary is stronger for small diffusion. As a result in a broad range of parameters a, q, P and b controlling mutual kinase–receptor

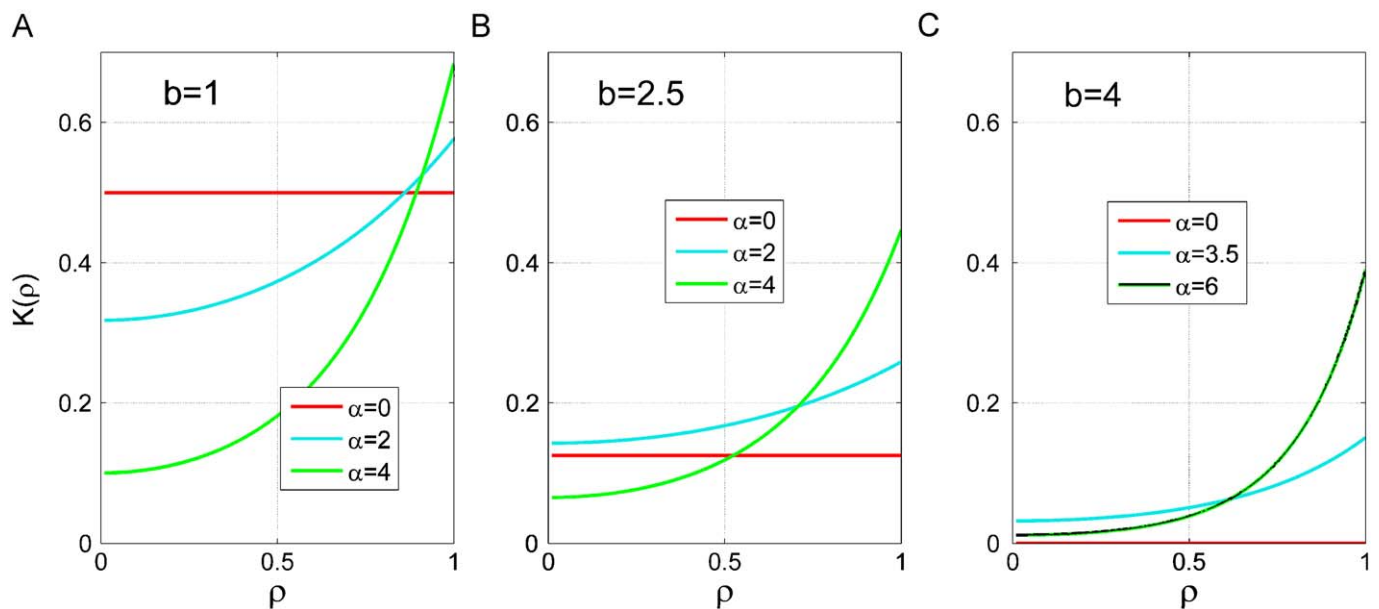


Fig. 2. The case with feedback. Profiles of active kinase concentration $K(\rho)$ for different values of $\alpha = d^{-1/2}$. Three qualitatively different cases corresponding to three different value of non-dimensional receptor dephosphorylation rate b are considered: Panel A ($b = 1, \alpha = 0, 2, 4$), Panel B ($b = 2.5, \alpha = 0, 2, 4$) and Panel C ($b = 4, \alpha = 0, 3.5, 6$). For all plots the remaining parameters are fixed: $q = a = P = 1$.

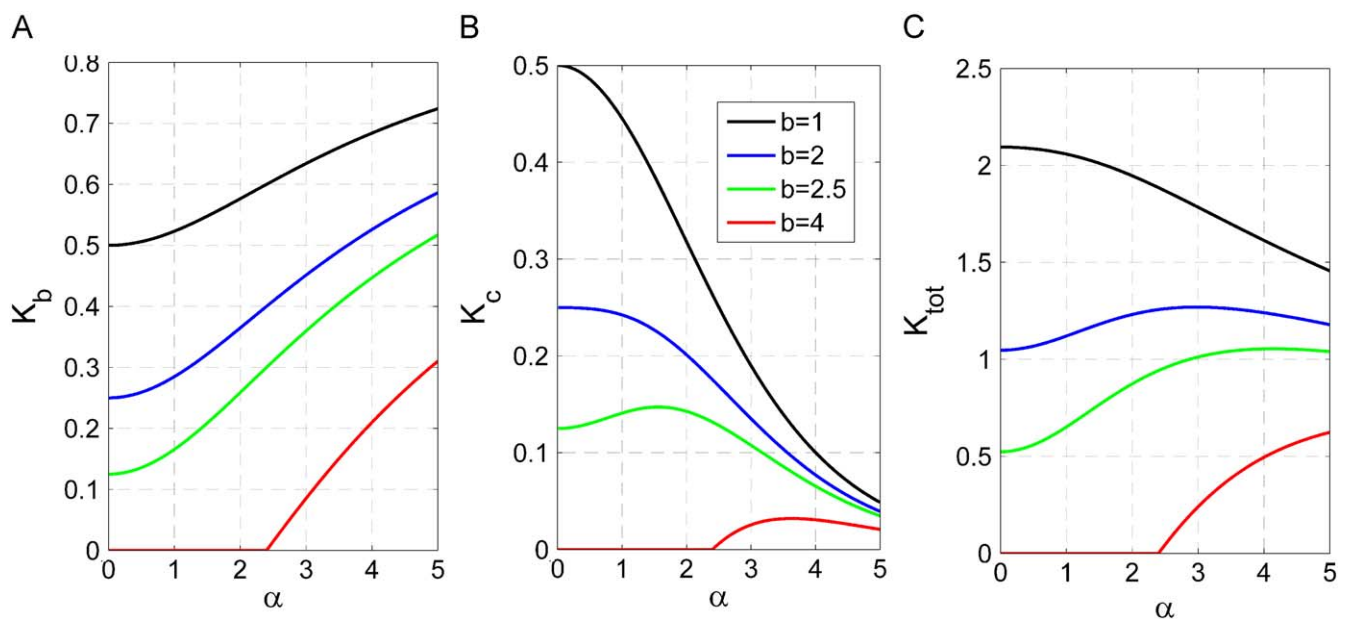


Fig. 3. The case with feedback. Concentration of active kinase at the boundary K_b (Panel A), in the cell center K_c (Panel B) and total amount of active kinase K_{tot} (Panel C) as a function of $\alpha = d^{-1/2}$. For all plots the remaining parameters are fixed: $q = a = P = 1$.

activation and inactivation rates the maximum value of the active kinase concentration in the cell center is reached for some finite value of the kinase diffusion coefficient. Moreover, for the large receptor inactivation rate ($b \geq 3qaP$) the active kinase concentration is everywhere zero for the infinite diffusion, but it is positive for the sufficiently small diffusion. Interestingly, for constant a , q and P , even for arbitrarily large inactivation coefficient b , there exists a positive steady state solution for the active kinase concentration if the diffusion is sufficiently small.

In living cells the diffusion and thus spatiotemporal localization of substrates can be controlled in a number of ways. Molecules can bind to a larger molecules of lower motility called buffers, or to the cell membrane and other structural elements directly or with help of the, so-called, anchoring proteins. On the

cell membrane receptors can form larger complexes of lower motility, or get localized within lipid rafts. Relevant to our model, cell membrane can create microdomains which trap signalling molecules, like Lck kinase, that activates TCR receptors (Douglass and Vale, 2005). Major simplification of our study is that it does not account for macromolecular crowding and presence of diffusion obstacles within the cell (organellen, cellular structures). The macromolecular crowding may have non-trivial effect on molecular association in the cell, possibly increasing its rate by limiting the volume in which molecules are free to diffuse (see Minton, 2001; Zimmerman and Minton, 1993).

Our simple model provides an example in which diffusion controls the strength of the feedback regulation and thus the dynamics of kinase activation. In the considered model, for a

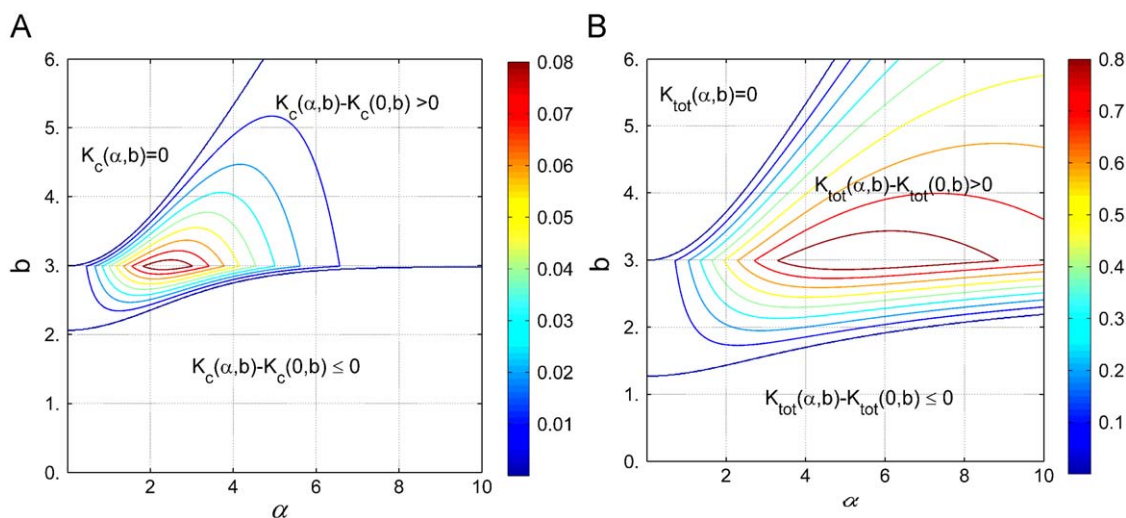


Fig. 4. The case with feedback. Dependence of active kinase concentration in the cell center (Panel A) and total amount of active kinase (Panel B) with respect to $\alpha = d^{-1/2}$ and non-dimensional receptor dephosphorylation rate b . In Panel A we may distinguish three domains in (α, b) plane in which respectively, $K_c(\alpha, b) = 0$; $\Delta_c = K_c(\alpha, b) - K_c(0, b) > 0$; $\Delta_c \leq 0$. In domain $\Delta_c > 0$, isolines of Δ_c are shown. Similarly, in Panel B we may distinguish three domains in which respectively, $K_{tot}(\alpha, b) = 0$; $\Delta_{tot} = K_{tot}(\alpha, b) - K_{tot}(0, b) > 0$; $\Delta_{tot} \leq 0$. In domain $\Delta_{tot} > 0$, iso lines of Δ_{tot} are shown. For both plots the remaining parameters are fixed: $q = a = P = 1$.

broad range of parameters the cell can be activated only when the kinase diffusion coefficient is sufficiently small, i.e. when reacting kinases and receptors are well colocalized that enable their mutual activation.

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Appendix A. Stability of spherically symmetric solutions

Let $\tilde{K}(\rho)$ and $\tilde{R} = const$ denote the spherically symmetric stationary solution of system (4)–(6). For the initial data not coinciding with $(\tilde{K}(\rho), \tilde{R})$, the solution $(K(x, \tau), R(\tau))$ to system (4)–(6) will be in general different, i.e.

$$K(x, \tau) = \tilde{K}(\rho) + \delta K(x, \tau), \quad R(x, \tau) = \tilde{R} + \delta R(x, \tau).$$

Our task here is to examine the asymptotic in time behavior of the functions K and R . We will confine here to initial data preserving the positivity of the functions K and R . The equations for δR and δK read

$$\frac{\partial \delta K}{\partial \tau} = d \nabla^2 \delta K - \delta K$$

inside the sphere and

$$\frac{d \delta R}{d \tau} = q \delta K_b (P - \tilde{R}) - (q \tilde{K}_b + b) \delta R - q \delta K_b \delta R,$$

$$a \delta R (1 - \tilde{K}_b) - a \tilde{R} \delta K_b - a \delta R \delta K_b = \mathbf{c} \mathbf{n} \cdot \nabla \delta K_b$$

on the sphere. Here we used the fact that \tilde{R} and \tilde{K} satisfy system (4)–(6). For $\varepsilon > 0$ an arbitrarily small positive number, let

$$\delta k = \delta K \exp \varepsilon \tau, \quad \delta r = \delta R \exp \varepsilon \tau. \quad (18)$$

Then, the above equations can be written as

$$\frac{\partial \delta k}{\partial \tau} = d \nabla^2 \delta k - (1 - \varepsilon) \delta k, \quad (19)$$

$$\frac{d \delta r}{d \tau} = q(P - \tilde{R}) \delta k_b - (q \tilde{K}_b + b + q \delta k_b \exp(-\varepsilon \tau) - \varepsilon \delta r, \quad (20)$$

$$a \delta r(x, \tau) (1 - \tilde{K}_b) = d \mathbf{n} \cdot \nabla \delta k + a(\tilde{R} + \delta r(x, \tau) \exp(-\varepsilon \tau)) \delta k_b. \quad (21)$$

The idea of the stability proof is to construct a time independent sub- and supersolution pairs: $(-\delta r_-, \delta k^-)$ and $(\delta r_+, \delta k^+)$. As $\varepsilon > 0$ this will prove that $\delta R(x, \tau)$ and $\delta K(x, \tau)$ tend to 0 as $\tau \rightarrow \infty$.

Let

$$\delta K^\pm(\rho) = \pm \delta K_{\pm b} \phi(\rho; \alpha_\varepsilon), \quad (22)$$

where $\alpha_\varepsilon = (1 - \varepsilon/d)^{1/2}$ and

$$\phi(\rho; \alpha) = \frac{(e^{\alpha \rho} - e^{-\alpha \rho})}{\rho(e^\alpha - e^{-\alpha})}. \quad (23)$$

Let us recall that ϕ is an increasing function of ρ and $\phi(1; \alpha) = 1$ for all $\alpha \in (0, \infty)$. In fact

$$\tilde{K}(\rho) = \tilde{K}_b \phi(\rho; \alpha_0). \quad (24)$$

Let

$$\delta K_{-b} = \tilde{K}_b - \psi(\varepsilon), \quad (25)$$

where $\psi(\varepsilon) \searrow 0$ as $\varepsilon \rightarrow 0$. This function will be specified later. It follows from (23) and (24) and the continuity of the function $\phi(\rho; \alpha_\varepsilon)$ with respect to the parameter ε that if $K(x, 0) > \eta > 0$ for $|x| \leq 1$, we can find $\varepsilon > 0$ so small that

$$K(x, 0) > \tilde{K}(\rho(x)) - \delta K_{-b} \phi(\rho(x); \alpha_\varepsilon) > 0$$

for all x inside the sphere. Let δK^+ be at least so large that $K(x, 0) < \tilde{K}(\rho(x)) + \delta K_{+b} \phi(\rho(x); \alpha_\varepsilon)$, implying that $\delta K(x, 0) < \delta K_{+b} \phi(\rho(x); \alpha_\varepsilon)$. Let

$$W_R = \frac{q(P - \tilde{R})}{q \tilde{K}_b - q \delta K_{-b} + b - \varepsilon} = \frac{q(P - \tilde{R})}{q \psi(\varepsilon) + b - \varepsilon}. \quad (26)$$

We will assume that

$$\delta R_\pm = \delta K_{\pm b} (W_R + \varepsilon). \quad (27)$$

Let us note that as

$$(P - \tilde{R})/\tilde{R} = b/(q \tilde{K}_b), \quad (28)$$

it follows from Eq. (27) that

$$\delta R_- = \tilde{R} - C_2 \psi(\varepsilon) + \tilde{C}_2 \varepsilon + o(\psi(\varepsilon)) + o(\varepsilon) \quad (29)$$

for some positive constants C_2 and \tilde{C}_2 . If, for small $\varepsilon \geq 0$, $\psi(\varepsilon)/\varepsilon \geq C_3 > 0$ with C_3 sufficiently large, then $\delta R_- < \tilde{R}$. On the other hand, for any $R(0) > 0$ we can find $\varepsilon > 0$ sufficiently small

such that $R(0) > \tilde{R} - \delta R_-$. Obviously, we may also choose δK_+ so large that $R(0) < \tilde{R} + \delta R_+$.

Let

$$\delta r_{\pm} = \delta R_{\pm}, \quad \delta k_{\pm b} = \delta K_{\pm b}. \quad (30)$$

Let $\delta k^{\pm}(\cdot)$ be constant in time spherically symmetric solutions to Eq. (19):

$$\delta k^{\pm}(\rho) = \pm \delta k_{\pm b} \phi(\rho; \alpha_{\varepsilon}). \quad (31)$$

We will prove that $(-\delta r_-, \delta k^-)$ and $(\delta r_+, \delta k^+)$ defined in (30) and (31) are, respectively, the sub- and supersolution pairs for system (19)–(21). So, after putting $\delta r = -\delta r_-$ in Eq. (20) we infer that the right hand side is positive if $\delta k_b \geq -\delta k_-$. In the similar way, after putting $\delta r = \delta r_+$ in Eq. (20) we infer that the right hand side is negative if $\delta k_b \leq \delta k_+$. It follows that if $\delta k_+ \geq \delta k(x, \tau) \geq -\delta k_-$ at the boundary, then $\delta r(x, \tau) \in (-\delta r_-, \delta r_+)$. We have thus to prove the corresponding properties of the functions δk^- and δk^+ with respect to Eqs. (19)–(21). First, as we said, the functions $\delta k^{\pm}(\rho)$ satisfy Eq. (19). So, to prove that δk^- , defined in (22) is a subsolution it suffices to show, as $(\delta k^-)'_B = w(1 - \varepsilon/d)(-\delta k_-)$, that for all $\delta r(x, \tau) \in (-\delta r_-, \delta r_+)$ we have

$$\delta r(x, \tau)(1 - \tilde{K}_b + \delta k_-) \geq -\tilde{R} \delta k_- - a^{-1} w \left(\frac{1 - \varepsilon}{d} \right) \delta k_-. \quad (32)$$

(See Pao, 1992, Chapter 2. Note that the coefficient by δk_b in (21) is positive.) Here

$$w(s) = \phi_{,\rho}(\rho = 1, s)$$

is smooth and monotonically increasing function of s for $s \geq (2d)^{-1}$. As $\delta k_- = \delta K_- < \tilde{K}_b$ then, putting $\delta r(x, \tau) = -\delta r_-$, we conclude that inequality (32) is implied by the inequality:

$$(1 - \tilde{K}_b + \delta K_-) \left[\frac{q(P - \tilde{R})}{q\tilde{K}_b - q\delta K_- + b - \varepsilon} + \varepsilon \right] < \tilde{R} + a^{-1} w \left(\frac{1 - \varepsilon}{d} \right),$$

where we used (27). Now, due to (28), $(P - \tilde{R})/\tilde{R} = b/(q\tilde{K}_b)$. Hence, we obtain the condition:

$$b(1 - \tilde{K}_b + \delta K_-)/(q\tilde{K}_b - q\delta K_- + b - \varepsilon) + O_1(\varepsilon) < \tilde{K}_b + \frac{\tilde{K}_b}{\tilde{R}} a^{-1} w \left(\frac{1 - \varepsilon}{d} \right).$$

As the spherically symmetric solution satisfies the equality

$$a\tilde{R}(1 - \tilde{K}_b) = w \left(\frac{1}{d} \right) \tilde{K}_b \quad (33)$$

then

$$b(1 - \tilde{K}_b + \delta K_-)/(q\tilde{K}_b - q\delta K_- + b - \varepsilon) + O_1(\varepsilon) < \tilde{K}_b + (1 - \tilde{K}_b) w \left(\frac{1 - \varepsilon}{d} \right) \left[w \left(\frac{1}{d} \right) \right]^{-1} \leq \tilde{K}_b + (1 - \tilde{K}_b)(1 - v(\varepsilon))$$

for some given smooth function v . Using (25) we arrive at the inequality

$$\frac{b(1 - \psi(\varepsilon))}{(q\psi(\varepsilon) + b - \varepsilon)} + O_1(\varepsilon) < 1 - (1 - \tilde{K}_b)v(\varepsilon).$$

This inequality can be satisfied if only $\psi(\varepsilon) \geq C(\varepsilon + v(\varepsilon))$ with $C > 0$ sufficiently large.

In the last step we have to prove that $\delta r(x, \tau)(1 - \tilde{K}_b) \leq (\tilde{R} + \delta r(x, \tau) \exp(-\varepsilon\tau))\delta k_+ + a^{-1} w(1 - \varepsilon/d)\delta k_+$ for all $\delta r(x, \tau) \in$

$(-\delta R_-, \delta R_+)$. As $\delta k_+ = \delta K_+ > 0$ and $\delta R_- < \tilde{R}$ then $\delta k_+(\tilde{R} + \delta r(x, \tau)) > 0$ for $\delta r(x, \tau) \in (-\delta R_-, \delta R_+)$. Thus we have only to prove that $\delta r(x, \tau)(1 - \tilde{K}_b) \leq a^{-1} w(1 - \varepsilon/d)\delta k_+$. Obviously, it suffices to show it for $\delta r = \delta r_+$. Let us recall that

$$\delta r_+ = \delta K_+(W_R + \varepsilon) = \delta K_+ \frac{q(P - \tilde{R})}{q\psi(\varepsilon) + b - \varepsilon} + \delta K_+ \varepsilon.$$

Hence we have to prove that

$$\left[\frac{q(P - \tilde{R})}{q\psi(\varepsilon) + b - \varepsilon} + \varepsilon \right] (1 - \tilde{K}_b) < a^{-1} w \left(\frac{1 - \varepsilon}{d} \right).$$

Using (28) and (33) we obtain as before that we have to satisfy the condition

$$\frac{b}{q\psi(\varepsilon) + b - \varepsilon} + O(\varepsilon) < \frac{w \left(\frac{1 - \varepsilon}{d} \right)}{w \left(\frac{1}{d} \right)}.$$

This is implied by the inequality:

$$\frac{b}{q\psi(\varepsilon) + b - \varepsilon} + O(\varepsilon) < 1 - v(\varepsilon),$$

where $v(\varepsilon) \rightarrow 0$ as $\varepsilon \rightarrow 0$. As before, this condition can be satisfied, if only $\psi(\varepsilon) \geq C(\varepsilon + v(\varepsilon))$ with $C > 0$ sufficiently large. Finally, taking into account what we have shown and using Theorem 2.1.2 from Pao (1992), we come to a conclusion that $-\delta r_- < \delta r(x, \tau) < \delta r_+$ and $\delta k^-(\rho(x)) \leq \delta k(x, \tau) \leq \delta k^+(\rho(x))$ for all $\tau \in (0, \infty)$.

So, due to definition (18) we conclude that $\delta K(x, \tau)$ and $\delta R(x, \tau)$ tend to zero in the supremum norm as $t \rightarrow \infty$.

We have thus shown that, if the initial data $K(x, 0)$ and $R(0)$ are positive, then the solution $(K(x, \tau), R(\tau))$ to system (4)–(6) tends to the unique spherically symmetric solution (\tilde{K}, \tilde{R}) as $\tau \rightarrow \infty$.

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