Qualitative Modelling of Quasi-homogeneous Effects in ERK and STAT Interaction Dynamics

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Abstract: *On the basis of qualitative analysis of author's model published in previous paper, the stability and temporal behaviour of quasi-homogeneous distributions of ERK-protein concentrations are analyzed in terms of corresponding reaction-diffusion problem. The stable quasi-homogeneous distributions are treated as a dynamical basis of pathway compartmentalization. It is also shown, that a crowding effect exists in the form of loss of pathway stability. An experimentally verifiable issue for possible existence of protein scaffolding mechanism is derived on the basis of its qualitative correspondence with the pattern formation and molecular crowding effects inherent to the considered model. Moreover, it is demonstrated, that the predicted ERK and STAT pathway instability can be interpreted as traveling wave propagation of molecular concentration drop and jump from the nucleus membrane to the cell one and vice versa.*

Keywords: *ERK- and STAT- interaction model, Quasi-homogeneous distributions.*

Introduction

There are two serious circumstances obligating the application of such named qualitative modeling (in the exact terms of dynamical systems theory) to the intracellular processes: Firstly, this is the well-known variability of cell responses to outer signals. Because of that, the measurements are not repetitive in time and ensemble of cells. Secondly, the specificity of intracellular processes is related to the essential role of regulatory processes assuring feedbacks in the cell. In this way, the non-linearity of the governing system of differential equations takes place. The exact (geometrical) language of the nonlinear equations theory is qualitative and very similar to the traditional biochemical one, being verbal and needing mathematical accuracy (in qualitative sense). Certainly, a similar approach requires verification of a qualitative correspondence between theoretically predicted effects and experimental concepts. For example, in this work we propose analogy between mathematically established crowding effect and a possible scaffolding mechanism to be experimentally searched.

In a previous author's paper [7], the dynamics of ERK- and STAT- protein interaction is modeled in a form of two-dimensional nonlinear dynamical system for the protein concentrations. Then the spatial modeling of the interaction is accomplished, by introducing an appropriate reaction-diffusion scheme. Here we consider this approach in a more specific way following the general methods of nonlinear dynamics as they are presented in [1, 5, 6]. Employing a reaction-diffusion equation, a corresponding problem is considered to describe both possible scenarios of compartmentalization and a molecular crowding effect in the form of concentration (density) drop and jump wave propagation of ERK-protein.

The author's model in [7] is based on the experimental results given in papers [8-9], where it is suggested that the STAT5 functional capacity of binding DNA could be influenced by the Mitogen-Activated Protein Kinase (MAPK)-pathway. Moreover, it is known that the serine phosphorylation of STAT 1 and 3 (Signal Transducers and Activators of Transcription) modulates their DNA-binding capacity and transcriptional activity. The interactions between STAT 5a and ERK1 and 2 (Extracellular signal-Regulated Kinases) are analyzed in paper [9]. The experiments (in vitro) have also showed, that the phosphorylation of the glutathione-S-transferase-fusion proteins using active ERK will occur, if only the fusion protein contains STAT5a sequence of wild-type. In addition, the other experiments with COS cells have demonsrated, that kinase-inactive ERK1 decreases GH stimulation of STAT5-regulated reporter gene of expression. These observations confirm that a direct physical interaction between ERK and STAT pathways exists for the first time. They identify also serine 780 as a target for ERK. These and other similar facts are taken into account to construct the model in paper [7].

The nonlinear distributed model of ERK and STAT interaction

Further, all considerations in this paper are qualitative in terms of the dynamical systems theory. The numerical values are dimensionless and have only illustrative sense in view of the absence of experimentally measured data on the subject. We firstly introduce the distributed nonlinear model of ERK and STAT interaction in the following form:

$$\frac{\partial \xi}{\partial t} = a\xi + b\eta + k_1 \xi \eta + D_\xi \frac{\partial^2 \xi}{\partial r^2},$$

$$\frac{\partial \eta}{\partial t} = c\xi + d\eta + k_1 \xi \eta + D_\eta \frac{\partial^2 \eta}{\partial r^2},$$
(1)

under boundary conditions

$$\left.\frac{\partial \xi}{\partial r}\right|_{\substack{r=0 \\ r=l}} = 0, \qquad \left.\frac{\partial \eta}{\partial r}\right|_{\substack{r=0 \\ r=l}} = 0,$$
(2)

taken from [7]. Here ξ and η are finite deviations (just new variables introduced but not small perturbations) of the STAT and ERK protein concentrations from the steady state values

$$e_2^0 = \left(k_3 s_2^0 + k_0 \Sigma\right)/k_2, \quad s_2^0 > 0,$$
(3)

obtained by equating to zero the right hand sides of the following model of ERK and STAT interaction with concentrated parameters (i.e. ordinary differential equations)

$$\frac{de_2}{dt} = k_1 ES - (k_1 S + k_2)e_2 - k_1 Es_2 + k_1 e_2 s_2,$$

$$\frac{ds_2}{dt} = -k_0 \Sigma + k_1 ES - k_1 Se_2 - (k_1 E + k_3)s_2 + k_1 e_2 s_2,$$
(4)

where e_2, s_2 are concentrations of ERK and STAT proteins respectively; k_1 is relatively small (with respect to $|a|$) coefficient, proportional to the frequency of collisions between ERK and STAT protein molecules and presents a rate constant of reactions of associations; k_2 and k_3 are constants of exponential growths and disintegrations; Σ is the sufficiently high

(to assure $s_2^0 > 0$) constant concentration of SOCS proteins, related to such named inhibitor source $I = k_0 \Sigma$ by the constant k_0; s_2^0 is the positive root of a quadratic equation also presented in [7]. Boundary $r = 0$ is situated at the nucleus membrane, and $r = l$ – at the cell membrane, respectively. The constants E and S are to the initial conditions of (4), described in [7]. In steady state approximation the model (1) takes the following form:

$$D_\xi \frac{d^2 \xi}{dr^2} = -a\xi - b\eta - k_1 \xi \eta ,$$

$$D_\eta \frac{d^2 \eta}{dr^2} = -c\xi - d\eta - k_1 \xi \eta .$$

(5)

Further we assume validity of the inequality $D_\xi << D_\eta$ in view of the fact that the ERK molecule is essentially smaller than STAT one [9]. Next we consider the first equation of (5) to be linear with respect to ξ and can be treated as an attached system in accordance with the Tichonov's theorem [14] (The theorem presents mathematical generalization of quasi-steady state and quasi-homogeneous (boundary layer) approximations). In our case we apply quasi-homogeneous approximation of steady state one of (1). For this purpose we take into consideration that η is sufficiently small "constant". Thus the attached system has a stable steady state of the center type (then well-known Lyapunov's definition of stability is satisfied). After replacing the homogeneous stationary value of ξ from the first equation in the second one (the degenerate system), the last one is obtained in the form

$$D_\eta \frac{d^2 \eta}{dr^2} = \frac{bk_1 \eta^2 + bc\eta}{a + k_1 \eta} - d\eta , \qquad \xi = -\frac{b\eta}{a + k_1 \eta} .$$

(6)

This equation is of spatial type (lack of time-dependence) and describes a quasi-homogeneous kinetics. Next the non-stationary kinetics, corresponding to (6), is described by the space-time equation in the form:

$$\frac{\partial \eta}{\partial t} = -\frac{bk_1 \eta^2 + bc\eta}{a + k_1 \eta} + d\eta + D_\eta \frac{\partial^2 \eta}{\partial r^2} ,$$

(7)

which is of reaction-diffusion type under boundary condition

$$\left. \frac{\partial \eta}{\partial r} \right|_{\substack{r=0 \\ r=l}} = 0 .$$

(8)

After developing the first two terms in the right hand side of (7) in a Taylor's series centered in $\eta = 0$ and retaining only the terms up to cubic power we obtain

$$\frac{\partial \eta}{\partial t} = \frac{bk_1^2}{a^3}(a - c)\eta^3 + \frac{bk_1(c - a)}{a^2}\eta^2 + \frac{ad - bc}{a}\eta + D_\eta \frac{\partial^2 \eta}{\partial r^2}$$

(9)

under the same boundary conditions (8). This cubic polynomial approximation means we accept a weak non-linearity (but not linearization) of the model (1), i.e. k_1 is sufficiently smaller than $|a|$ or k_2, k_3 in order to assure the approximation validity. The last inequalities

follow from the biochemical consideration that the processes of ERK inactivation and STAT dephosphorylation are faster than that of ERK and STAT interaction. The last process is of molecular recognition type [9].

Stability analysis of the quasi-homogeneous distribution of ERK protein

Let us now substitute in (9) the perturbation solution $\eta(t,r) = \overline{\eta}(r) + \omega(t,r)$, where $\overline{\eta}(r)$ is a quasi-homogeneous steady state solution of (7) and $\omega(t,r)$ is a small variation (perturbation). As a result we obtain the next variation equation

$$\frac{\partial \omega}{\partial t} = \left\{ \frac{3bk_1^2}{a^3}(a-c)\overline{\eta}^2 + \frac{2bk_1(c-a)}{a^2}\overline{\eta} + \frac{ad-bc}{a} \right\}\omega + D_\eta \frac{\partial^2 \omega}{\partial r^2}, \tag{10}$$

under initial condition (playing the role of a dissipative structure in this case)
$$\omega(0,r) = \varphi(r), \tag{11}$$

and boundary conditions

$$\frac{\partial \omega}{\partial r}\bigg|_{\substack{r=0 \\ r=l}} = 0. \tag{12}$$

By applying the standard procedure similar to that in the author's paper [6], the solution of (10) can be obtained in the form

$$\omega(t,r) = \sum_{n=0}^{\infty} a_n e^{Q(\overline{\eta})t} \cos\sqrt{\lambda_n}\, r, \tag{13}$$

where

$$a_n = \frac{2}{l}\int_0^l \varphi(r)\cos\sqrt{\lambda_n}\, r\, dr, \qquad \lambda_n = \left(\frac{n\pi}{l}\right)^2, \tag{14}$$

$$Q(\overline{\eta}) = \frac{3bk_1^2}{a^3}(a-c)\overline{\eta}^2 + \frac{2bk_1(c-a)}{a^2}\overline{\eta} + \frac{ad-bc}{a} - D_\eta\lambda_n. \tag{15}$$

Next we denote

$$\frac{3bk_1^2}{a^3}(a-c) = -\theta, \qquad \frac{2bk_1(c-a)}{a^2} = -\tau, \qquad \frac{ad-bc}{a} - D_\eta\lambda_n = -\gamma, \tag{16}$$

where θ, τ, γ are positive numbers in view of the relations
$$a < 0, \quad b < 0, \quad c < 0, \quad d < 0, \quad D_\xi << D_\eta, \quad c-a = k_3 > 0, \tag{17}$$

following from [7] at the absence of non-interacting ERK proteins. Then the expression (15) takes the form

$$Q(\overline{\eta}) = -\theta\overline{\eta}^2 - \tau\overline{\eta} - \gamma = -\theta(\overline{\eta} - \overline{\eta}_1)(\overline{\eta} - \overline{\eta}_2) \tag{18}$$

Here

$$\overline{\eta}_{1,2} = \frac{1}{2\theta}\left(-\tau \pm \sqrt{\tau^2 - 4\theta\gamma}\right) \qquad (19)$$

are the roots of the quadratic polynomial $Q(\overline{\eta})$. There are two negative steady state values of the deviation $\overline{\eta}$ from the steady state value of the concentration e_2^0 assumed to be larger than the corresponding deviations. It is easy to show that $Q(\overline{\eta})$ will be negative when the steady state concentration is out of the interval between the two roots mentioned. In this case the perturbation solution (13) will attenuate and the dissipative structure (11) wiil be stable, thus it could really exist. For a steady state deviation smaller than the bigger root and larger than the smaller one, however, $Q(\overline{\eta})$ will be positive if the structural wave number λ_n or diffusion coefficient D_η is sufficiently small. It means that the dissipative structure (11) will be unstable and will disappear in this case. Thus too low and too high steady state concentrations are indicative for the dissipative structures existence (i.e. – compartmentalization). On the contrary, the similar structures are not observed for the average ones. In this sense, the well-known crowding effect [12] could be unambiguously interpreted as a case $\eta_1 + s_2^0 \approx 0$, when only the increase of steady state distribution $\overline{\eta}(r)$ could assure dissipative structure stability in the cell.

Hypothetical mechanism of STAT scaffolding ERK pathway

As it was shown in the previous section, too low and too high steady state concentrations are indicative for the dissipative structures existence, but the average ones are not. Following this basic conclusion, in this section it will be shown how a possible scaffolding effect [10, 11, 13] can be related to the described behavior of η (activated ERK). In terms of the stability analysis, described above the magnitude of an initial disturbance η of activated ERK depends critically on its own value. It is well known that the corresponding initial values of η can amplify or attenuate in a regime of instability or stability, respectively. The dynamical interpretation of ERK criticality consists in the effect above theoretically established, i.e. the ERK pathway is unstable in some interval of ERK concentration. That means that the initial concentration of ERK, belonging to this interval, does not conserve its amplitude, i.e. it will amplify. Thus ERK pathway is sensitive at intermediate concentrations of ERK. Out of this interval of average ERK concentrations, however, the ERK pathway becomes insensitive, i.e. the distribution conserves its magnitude. This purely qualitative consequence from our model can be explained physically by hypothetical STAT scaffolding mechanism of ERK signaling, presented in Figs. 1 and 2. From a biological point of view, the scaffold is a protein, which main function, is to bring other protein in order to interact together for them. Such a protein usually has many protein binding domains. Unfortunately these specific domains have not established for STAT yet. But in the basic work [9] still has mentioned about "unknown region on STAT5a". Concerning that we accept the hypothesis that STAT may be has several binding domains and we try to draw some conclusions from this assumption. In papers [2, 4] the corresponding insights into this hypothesis have provided through computer simulations of signaling pathways with scaffolds in general sense. On the basis of these studies our first idea, related to STAT scaffolding mechanism, is presented in Fig. 1. It illustrates the principle of balance: Adding too much ERK concentration we can decrease the output of ERK scaffolding cascade, just as adding too much scaffold STAT (Fig. 2). The analogy of the presented simple mechanism with the dynamical behaviour of ERK signaling is evident: In the both cases the ERK pathway will amplify a signal for intermediate concentration of

scaffold STAT and will not amplify it for low and high concentrations. In Fig. 2 it is seen again a scheme like combinatorial inhibition. In the same way, the signaling down scaffolding ERK cascade is a question of balance: If there is too small STAT concentration, ERK signaling will be low (left). At an intermediate STAT concentration, the ERK signaling will be high (center). Once the STAT concentration exceeds that of the ERK it will bind and the signaling begins to decrease (right).
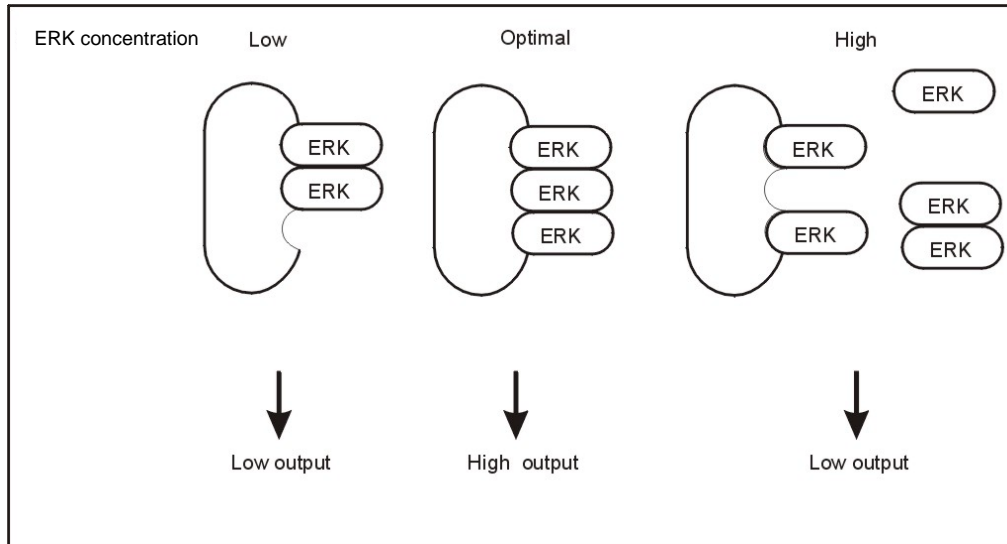

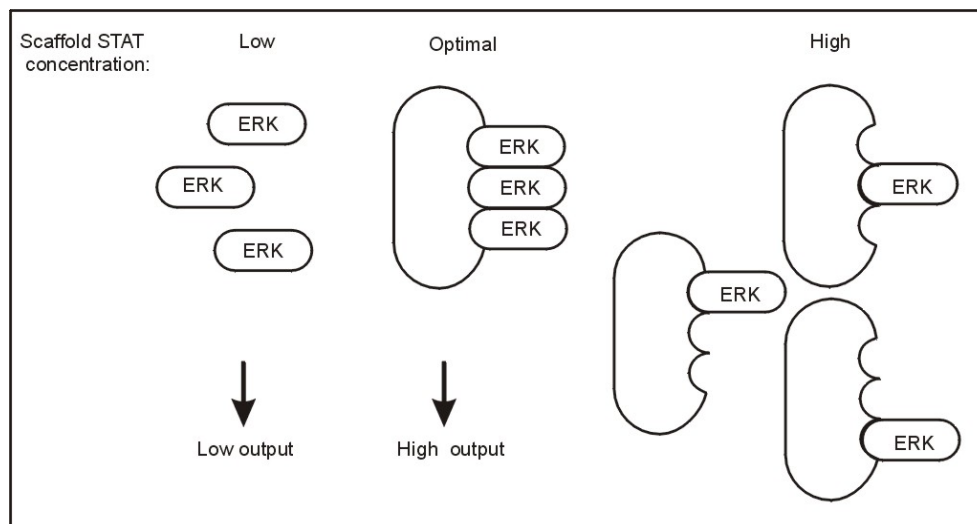Fig. 1 Dependence of ERK signaling on ERK concentration

Fig. 2 Dependence of ERK signaling on STAT concentration

The most important question now is whether ERK and STAT interaction really exhibits the scaffold mechanism predicted in this section? Excepting the unknown number of binding domains of scaffold STAT, needy of measures, there are more experimental evidences for the hypothetical mechanism, suggested here, than principal objections against it.

Density drop and jump propagation in the non-stationary quasi-homogeneous model of ERK- and STAT- protein interaction

The Eq. (9), presenting non-stationary quasi-homogeneous kinetics, can be written in the form:

$$\frac{\partial \eta}{\partial t} = -\alpha \eta^3 - \beta \eta^2 - \gamma \eta + D_\eta \frac{\partial^2 \eta}{\partial r^2} = -\alpha \eta (\eta - \eta_1)(\eta - \eta_2) + D_\eta \frac{\partial^2 \eta}{\partial r^2}, \tag{20}$$

where

$$\frac{bk_1^2}{a^3}(a - c) = -\alpha, \qquad \frac{bk_1(c - a)}{a^2} = -\beta, \qquad \frac{ad - bc}{a} = -\gamma \tag{21}$$

and α, β, γ are positive values. Moreover, the roots η_1, η_2, η_3 of the corresponding cubic polynomial can be given by the formula

$$\eta_{1,2} = \frac{1}{2\alpha}\left(-\beta \pm \sqrt{\beta^2 - 4\alpha\gamma}\right), \qquad \eta_3 = 0, \tag{22}$$

We assume again the steady state value of the concentration e_2^0 to be larger than the corresponding deviations η. It can be shown that in the homogeneous case (the diffusion term equals zero), the smaller root η_1 is asymptotically stable, the bigger η_2 is unstable, and the zero one η_3 is indifferent (i.e. – stable by Lyapunov definition but not asymptotically). Thus the lowest steady state value of the ERK concentration is practically realized in the homogeneous case, corresponding to the absence of appropriate nucleus response in the form of STAT-protein production, initiated by cell signaling. When similar response (STAT-protein increase) is available, the ERK concentration dramatically increases near the nucleus membrane and some quasi-homogeneous radial distribution of ERK concentration takes place in the cytosol. That means that the diffusion involves in the process and the equation (20) becomes valid. Moreover, it can be transformed in the ordinary differential equation of second order

$$D_\eta \eta'' \pm v \eta' - \alpha \eta^3 - \beta \eta^2 - \gamma \eta = 0, \tag{23}$$

and next – in the following system of differential equations of first order

$$\eta' = x, \qquad x' = \frac{1}{D_\eta}\left(\pm vx + \alpha \eta^3 + \beta \eta^2 + \gamma \eta\right), \tag{24}$$

where $\eta = \eta(r \pm vt)$ is a traveling wave function of some density (concentration) jump propagation, which will be determined. For the purpose we firstly choose the wave propagates along the positive direction of axis r, i.e. – from the nucleus membrane to the cell one. The system (24) has the same steady state values for η as in the homogeneous case, considered above. However, in the quasi-homogeneous case, presented by (23) or (24), the less root η_1 is unstable, the bigger η_2 is stable, and the zero one η_3 is unstable. Thus, in quasi-homogeneous case the steady state values change the type of their character in comparison with the homogeneous one. The ERK concentration will increase from the destabilized value η_1 to stabilized one η_2. A density (concentration) jump will propagate from the nucleus to cell membrane in view of the fact that $\eta_2 > \eta_1$ (Fig. 3).
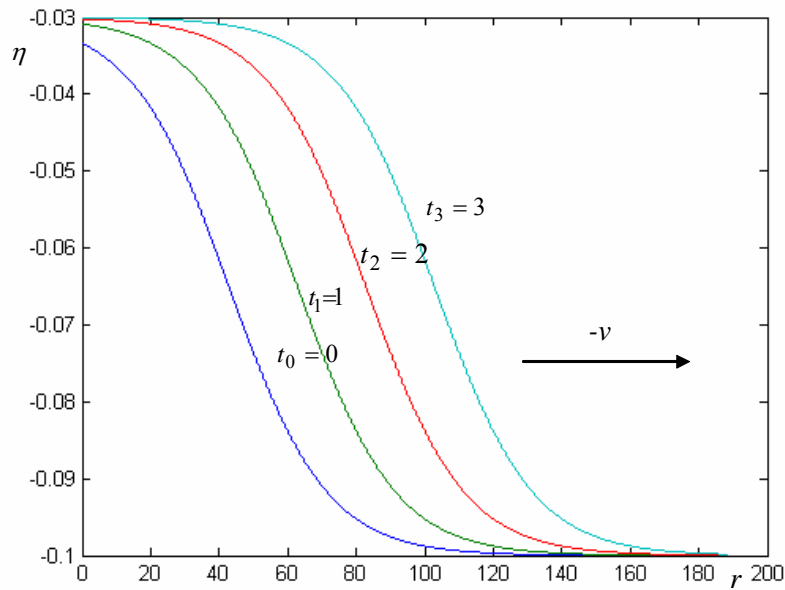
Fig. 3 Curve form propagation of the ERK density jump
at time scale $t_i = t - 5, \quad i = 0, 1, 2, 3$

Once the higher steady state value η_2 is realized everywhere in the cytosol along the axis r as a response of cell signaling, the model will become homogeneous again (as it was before the response) and η_1 will stabilize (as well as η_2 will lose stability). Then it is reasonable to expect that along time (i.e. in terms of the homogeneous case) and near the cell membrane the density η will change from unstable value η_2 to stable one η_1. This change is termed as concentration (density) drop having in view that $\eta_2 > \eta_1$. In terms of the quasi-homogeneous case, the domain of density near the lower value η_1 will propagate from the cell membrane to cell nucleus and the higher density region will become more and more narrow. That is the density drop will propagate in the opposite direction, i.e. – from the cell membrane to the nucleus (Fig. 4). In fact the density drop is also opposite to the direction of stabilization (it occurs from the higher stable η_2 to lower unstable on η_1), what needs additional maintenance in the form of new signal (ligand) at the membrane to inhibit ERK activation. For example RKIP can indirectly play a similar role by inhibiting the phosphorylation and activation of MEK by Raf-1 and then ERK. As a result the process of density drop conveys cell signaling to initiate response of the nucleus, described above. In this way ERK periodic activations take place. On the contrary, the ERK concentration will move to the less stable value η_1 relatively much slower at the absence of RKIP influence. That is, ERK will remain activated for longer period without RKIP presence. Thus RKIP should augment the frequency of ERK periodic activations. The proposed drop and jump mechanism of signaling and response transduction respectively, needs solving appropriate wave problem, which is proposed in the next section.
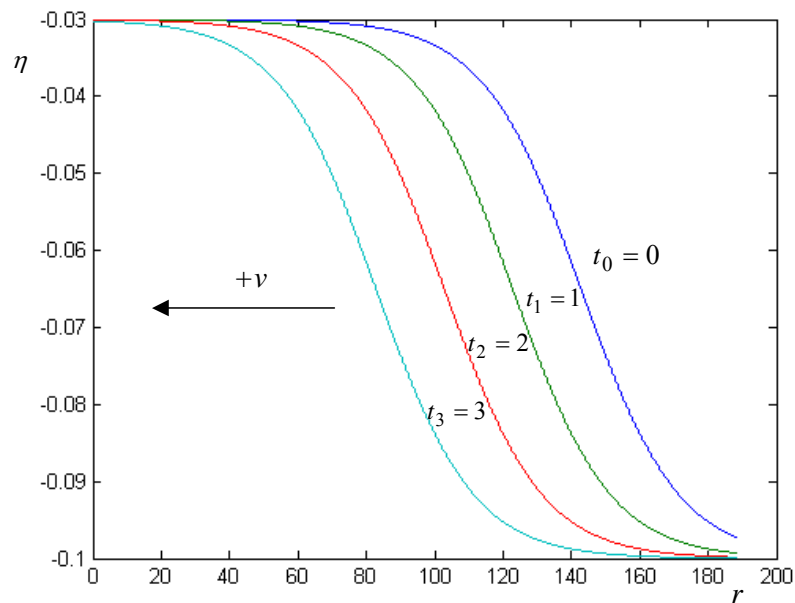
Fig. 4 Curve form propagation of the ERK density drop
at time scale $t_i = t + 10, \quad i = 0, 1, 2, 3$

Propagation velocity and curve form of the ERK density drop and jump

Let us assume the notation $\eta' = G(\eta)$. Then $\eta'' = G'(\eta)\eta'$. While we need a drop and jump connecting the two steady state values η_1 and η_2 we also assume

$$G(\eta) = \eta' = k(\eta - \eta_1)(\eta - \eta_2), \tag{25}$$

where k is a constant, which can be determined. The differentiation of (25) yields

$$\eta'' = k^2(\eta - \eta_1)(\eta - \eta_2)(2\eta - \eta_1 - \eta_2), \tag{26}$$

which can be substituted in the model equation (23) to write the identity

$$D_\eta k^2 (2\eta - \eta_1 - \eta_2) \pm kv - \alpha\eta = 0. \tag{27}$$

By equating to zero the coefficient in the front of the variable η and the free term in (27) we obtain the formulas

$$k = \sqrt{\frac{\alpha}{2D_\eta}}, \tag{28}$$

and

$$v = \pm(\eta_1 + \eta_2)\sqrt{\frac{\alpha D_\eta}{2}} > 0, \eta_1 + \eta_2 < 0. \tag{29}$$

By combining (25), (28) and integrating the obtained equation, it follows

$$\eta = \frac{\eta_1 + \eta_2}{2} + \frac{\eta_1 - \eta_2}{2} th\left\{-\frac{1}{2}\left[k(\eta_1 - \eta_2)(r \pm vt)\right] + a\right\}, \quad a = \ln(-A), \qquad (30)$$

where A is integration constant and th is the notation of hyperbolic tangent. The formula (30) presents a wave solution of the reaction-diffusion model (25) in the form of density drop and jump propagation with a velocity (29). It is valid for the case when $\eta \in (\eta_1, \eta_2)$. Curve forms of type (30) are presented in Fig. 3 for time moments 0, 1, 2, 3. The exemplary values of parameters are $v = \pm 20$, $k = 1$, $a = 2$, $\eta_1 = -0.1$, $\eta_2 = -0.03$. It is seen that we can expect a concentration drop and jump effect only for average concentration of ERK protein (i.e. – in the interval $(-0.1, -0.03)$. Moreover, the formulas (28), (29) and (30) can be used to analyze possible experimental data of ERK and STAT interaction in the intracellular space.

From the practical point of view, it is of interest to note that on the basis of developed theory (formulas (28), (29), (27), (26)) and by taking into account the necessary formulas for a, b, c, from [7]) the velocity v of density drop and jump propagation can be expressed in the form:

$$v = \sqrt{\frac{D_\eta k_1 k_3 (S - s_2^0)}{2\left[k_1 (E - e_2^0) + k_3\right]}}, \qquad (31)$$

where all values, involved in the right hand side, are experimentally measurable. Therefore, if these values are known, a realistic value of velocity v could be numerically estimated.

On the practical issues of qualitative modeling ERK and STAT interaction

This paper is a continuation of previous author's paper [6], where it is shown that the distributed mathematical models of MEK/ERK- and JAK/STAT-pathways, separately considered, have stable homogenous steady states. Thus, we could not expect instability in view of the fact that no autocatalytic reaction (activator) is presented both in MEK/ERK- and JAK/STAT-pathways, separately considered. In order to explain the non-homogenous distributions of the mentioned pathways, it is necessary to search instability in the ERK and STAT interaction. The dynamical model of reaction-diffusion interaction of ERK and STAT proteins is presented in another author's paper [7]. Here we analyze qualitatively this model to demonstrate the effects of compartmentalization and concentration drop and jump can be simulated as quasi-homogeneous distributions of protein concentrations (densities) in our case. This is a qualitative indication that modeling signaling pathway with reaction-diffusion equations affords a realistic approach for the analysis of spatially restricted biochemical reactions in cell signaling. Certainly, in view of the fact that the modeling parameters are usually gathered from biochemical experiments on purified components while functional effects arise from cell physiological experiments, one does not aim numerical (i.e. quantitative) agreement between experimental data of concentration drop and jump effect and some modeling prediction. Instead, the modeler should aim for qualitative scaling relationship as it is done in *Section 2* of this paper by applying Tichonov's theorem. The gradual refinement of a corresponding dynamical model (1) is only an example of one iterative process. Once the time scale of activation and deactivation processes of ERK and STAT interaction is correctly established, one could investigate the dynamical behaviour, including possible existence of compartmentalization, i.e. pattern formation, crowding effect, concentration drop and jump wave propagation. The use of similar analysis would be in a possible confirmation or rejection a scaffolding mechanism to be established experimentally

in this case. Here the practical issue is concentrated on the question, put in *Section 4*: Whether ERK and STAT interaction really exhibits the scaffold mechanism predicted in this paper? The existence of this mechanism can be experimentally verified. It would be a main contribution of the model presented in [7] to the biochemical practice. It seems quite realistic, that in a cell level the most signaling networks of protein interactions contain scaffolding mechanism. On the other hand, if the stability analysis predicts that some components (concentrations) are sensitive to small perturbations in model parameters, this would not necessarily mean that the model will be incorrect. As it is shown in *Section 3*, it could suggest that the components of ERK and STAT interaction model might be susceptible to external perturbation, and an effect of molecular crowding type could take place.

Conclusion

The present qualitative analysis shows that diffusion (together with the corresponding biochemical reactions) is likely to play a critical role in governing the space-temporal behaviour of ERK and STAT interaction system and should not be ignored. It is an essential part of cellular complexity inherent to space-temporal description. A new generation of microscopic techniques, capable of resolving the intracellular localization of proteins, would provide evidence of the experimental availability of pathway compartmentalization, molecular crowding and drop and jump propagation. Computer simulation of physics-based models, coupled with quantitative space-temporal data would allow to cell biologists to develop and test the adequate hypothesis of dynamical nature of the effects, above discussed.

Acknowledgements

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