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Influence of RKIP Protein Complexes Concentrations on the Quasi-Stationary Behaviour of the MEK/ERK Signal Transduction Pathway

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Summary: It is considered the quasi-stationary approximation of a dynamic model, representing a positive feedback mechanism in the MEK/ERK signaling pathway mediated by RKIP. An analytical solution of the eight dimensional quasi-stationary system is found. Analytical relations between stationary and initial values of all signal pathway components are derived too. On the basis of them it is established that the quasi-stationary behaviour of a MEK/ERK pathway is dependent only on reactions of association and dissociation of the double phosphorylated protein ERKpp and the protein complex RKIP/Raf-1 to the three-component complex ERKpp/RKIP/Raf-1 and vice versa. Moreover, the leading role of initial concentrations of the RKIP protein complexes is determined.

Keywords: MEK/ERK Pathway, Quasi-stationary Approximation, Analytical Relations.

1. INTRODUCTION

The Ras/Raf/MEK/ERK signaling pathway (marked as a MEK/ERK pathway throughout the remainder of this paper) is a mitogen-activated protein kinase (MAPK) pathway, which exists in most, if not all eukaryotic cells, and is involved in various biological responses. For example it controls fundamental processes and is often deregulated in human cancer [1-5]. In view of the great biological significance of this biochemical phenomenon the scientists consider different hypotheses, related to its reaction mechanism, which subsequently are accepted or rejected by theoretical or experimental verification. For example in [6] the influence of the protein RKIP on the MEK/ERK signaling pathway is discussed. In [7] the hypothesis for existence of a positive feedback mechanism in the MEK/ERK dynamics mediated by RKIP is considered. In the both papers experimental investigations have

described the role that the initial RKIP protein concentration plays on the pathway activity. On the other hand in the author papers [8-11] it is proved that the influence of the initial RKIP concentration on the MEK/ERK pathway activity ceases near to its quasi-stationary state. Moreover, the pathway steady state already depends on initial values of other pathway components. In this way the task of this investigation is to develop further the results, obtained in [11] in order to draw additional information for the MEK/ERK signal trasduction process.

2. A QUASI-STATIONARY APPROXIMATION OF THE MEK/ERK SIGNALING PATHWAY DYNAMICAL MODEL

As a result of applying of the Tichonov's theorem, in [11] the original seventeen dimensional dynamical model of a MEK/ERK signaling pathway with a positive feedback mechanism is reduced to the following eight dimensional quasi-stationary approximations:

$$\frac{dm_2}{dt} = k_3 m_3 - k_6 m_6 \tag{2.1}$$

$$\frac{dm_3}{dt} = -k_3 m_3 + k_6 m_6 \tag{2.2}$$

$$\frac{dm_4}{dt} = k_3 m_3 - k_{17} m_{16} \tag{2.3}$$

$$\frac{dm_6}{dt} = k_{17} m_{16} - k_6 m_6 \tag{2.4}$$

$$\frac{dm_7}{dt} = k_6 m_6 - k_{17} m_{16} \tag{2.5}$$

$$\frac{dm_{13}}{dt} = -k_{15} m_{13} m_{15} + (k_{16} + k_{17}) m_{16} \tag{2.6}$$

$$\frac{dm_{15}}{dt} = -k_{15} m_{13} m_{15} + (k_{16} + k_{17}) m_{16} \tag{2.7}$$

$$\frac{dm_{16}}{dt} = k_{15} m_{13} m_{15} - (k_{16} + k_{17}) m_{16} \tag{2.8}$$

where $m_2, m_3, m_4, m_6, m_7, m_{13}, m_{15}$ and m_{16} are state variables representing concentrations of the protein complexes SOS/Grb2 and

SOS/Grb2/Ras, the activated protein Ras*, the protein complex Ras/Raf-1, the activated protein Raf-1*, the double phosphorylated protein ERKpp and the protein complexes RKIP/Raf-1 and ERKpp/RKIP/Raf-1, respectively. Moreover in accordance with the Tichonov's terminology [12] the last ones are slow varying protein concentrations in the MEK/ERK signaling pathway (the fast variables of the original MEK/ERK pathway model are not presented in (2.1) - (2.8) in view of the quasi-steady state approximation). In [11] it is shown that the quasi-stationary transduction process is dependent on the behaviour of some explicit slow variables, forming the three-dimensional dynamic system (2.6) - (2.8). Moreover in the same paper the leading role of the system variable m_{16}, representing dynamics of the ERKpp/RKIP/Raf-1 protein complex is established. In view of the fact, that this variable takes part in the right hand sides of the equations of a quasi-stationary system (2.1) - (2.8), excepting the first two of them, it was considered as a "driver" of the MEK/ERK pathway nearly its quasi-stationary state. In addition in [11] the type of behaviour of this "driver" is specified. The stable ERKpp/RKIP/Raf-1 concentration was numerically determined (lower or around $0.0224\,\mu M$) at which stabilization of the MEK/ERK quasi-stationary process could be observed. Here in order to further investigation of the quasi-stationary system (2.1) - (2.8), firstly we will focus on the established algebraic relations between its components. The last ones are derived as a result of adding of the several system equations and can be written in the following manner:

$$m_2 + m_3 = A_1, \quad m_3 + m_4 + m_6 = A_2, \quad m_6 + m_7 = A_3, \\ m_{13} + m_{16} = A_4, \; m_{15} + m_{16} = A_5 \tag{2.9}$$

where $A_i (i = 1,2,..,5)$ are constants, determined by the initial protein concentrations of a MEK/ERK pathway. In accordance with the quasi-stationary approximation considered here the expressions (2.9) include only slow varying protein concentrations of the transduction pathway. Moreover the same ones will be valid only when the signaling pathway approaches near to its steady state.

3. ANALYTICAL RELATIONS BETWEEN INPUT AND OUTPUT SIGNALS IN THE MEK/ERK SIGNAL TRANSDUCTION PATHWAY

In this paragraph we will prove direct dependence of the quasi-stationary transduction process on the type of dynamical behaviour only of one of its signal components. For the purpose the quasi-stationary system (2.1) - (2.8) is analytically solved. Further by considering its solution in infinity the following stationary values (denoted by upper index "0") of the slow varying protein concentrations in the MEK/ERK pathway are derived:

$$m_2^0 = A_1 - \frac{k_{17}}{k_3} m_{16}^0 \tag{3.1}$$

$$m_3^0 = \frac{k_{17}}{k_3} m_{16}^0 \tag{3.2}$$

$$m_4^0 = A_2 - \frac{k_{17}(k_3 + k_6)}{k_3 k_6} m_{16}^0 \tag{3.3}$$

$$m_6^0 = \frac{k_{17}}{k_6} m_{16}^0 \tag{3.4}$$

$$m_7^0 = A_3 - \frac{k_{17}}{k_6} m_{16}^0 \tag{3.5}$$

$$m_{13}^0 = A_4 - m_{16}^0 \tag{3.6}$$

$$m_{15}^0 = A_5 - m_{16}^0 \tag{3.7}$$

where

$$A_1 = m_2^i + m_3^i \tag{3.8}$$

$$A_2 = m_3^i + m_4^i + m_6^i \tag{3.9}$$

$$A_3 = m_6^i + m_7^i \tag{3.10}$$

$$A_4 = m_{13}^i + m_{16}^i \tag{3.11}$$

$$A_5 = m_{15}^i + m_{16}^i \tag{3.12}$$

Here $m_j^i\,(j=2,3,4,6,7,13,15,16)$ are initial slow varying protein concentrations of the MEK/ERK pathway. In addition on the basis of the results, obtained in [11] analogous analytical relations can be found for the fast varying components of the original MEK/ERK pathway model too. They can be written in the following manner:

$$m_1^0=\frac{k_{17}(k_2+k_3)m_{16}^0}{k_1(k_3A_1-k_{17}m_{16}^0)} \tag{3.13}$$

$$m_5^0=\frac{k_{17}(k_5+k_6)m_{16}^0}{k_4(k_3k_6A_2-k_{17}(k_3+k_6)m_{16}^0)} \tag{3.14}$$

$$m_8^0=\frac{k_6k_{17}(k_8+k_9)m_{16}^0}{k_7k_9(k_6A_3-k_{17}m_{16}^0)} \tag{3.15}$$

$$m_9^0=\frac{k_{17}m_{16}^0}{k_9} \tag{3.16}$$

$$m_{10}^0=\frac{k_{17}(k_{11}+k_{12})m_{16}^0}{k_7k_9m_{11}^0} \tag{3.17}$$

$$m_{11}^0=\frac{k_{17}(k_{11}+k_{12})m_{16}^0}{k_7k_9m_{10}^0} \tag{3.18}$$

$$m_{14}^0=\frac{k_4k_{14}(A_5-m_{16}^0)(k_3k_6A_2-k_{17}(k_3+k_6)m_{16}^0)}{k_3(k_5+k_6)k_{17}m_{16}^0} \tag{3.19}$$

$$m_{17}^0=\frac{k_{17}m_{16}^0}{k_{18}} \tag{3.20}$$

where $m_1^0, m_5^0, m_8^0, m_9^0, m_{10}^0, m_{11}^0, m_{12}^0, m_{14}^0$ and m_{17}^0 are stationary concentrations of the proteins Ras, Raf-1 and MEK, the protein complex Raf-1*/MEK, the double phosphorylated protein MEKpp, the protein ERK, the protein complex MEKpp/ERK, the protein RKIP and phosphorylated protein RKIPp, respectively. It is seen that the stationary value of m_{16} takes part in the right hand sides of the formulas (3.1) - (3.7). It confirms the conclusion mentioned in the previous paragraph that the stable character of this slow varying signal component appears to be a sufficient condition for stabilization of the other components of the quasi-stationary system (2.1) - (2.8). On the other hand the presence of the same variable in

right hand sides of the formulas (3.13) - (3.20) shows that the type of dynamical behaviour of the complex ERKpp/RKIP/Raf-1 will determine the quasi-stationary state of the MEK/ERK pathway as a whole. This means that all the output signals of the pathway will be stabilized, if the concentration of the ERKpp/RKIP/Raf-1 protein complex is lower or around $0.0224\,\mu M$. In addition, by the analytical solution of the system (2.1) - (2.7) the stationary value of the ERKpp/RKIP/Raf-1 protein complex is determined, as it follows:

$$m_{16}^{0} = \frac{\sqrt{\Delta} - D_1}{\sqrt{\Delta} + D_2} \tag{3.21}$$

where

$$\Delta = \frac{1}{4}(k_{15}A_4 + k_{15}A_5 + k_{16} + k_{17})^2 - k_{15}^2 A_4 A_5 \tag{3.22}$$

$$D_1 = \frac{1}{2}(k_{15}A_4 + k_{15}A_5 + k_{16} + k_{17}) - k_{15}A_4A_5 \tag{3.23}$$

$$D_2 = \frac{1}{2}(k_{15}A_4 + k_{15}A_5 + k_{16} + k_{17}) - k_{15} \tag{3.24}$$

The expressions (3.22) - (3.24) show that besides of its own initial value the type of behaviour of the ERKpp/RKIP/Raf-1 concentration is dependent on the initial values of its forming elements (i.e. of the double phosphorylated protein ERKpp and the protein complex RKIP/Raf-1) and the corresponding rate constants of its association and dissociation (k_{15}, k_{16} and k_{17}) as well. This means, that by changing m_{13}^{i}, m_{15}^{i} and m_{16}^{i} we can essentially control (at least theoretically) the steady state behaviour of the whole signaling process. Moreover it will be of interest to see how the type of pathway behaviour could be changed (from stable toward unstable and vice versa), if there are small fluctuations of biochemical reaction rates k_{15}, k_{16} and k_{17}. For the purpose the bifurcation analysis must be carried out. It will be a basic task, however, for our future investigation. On the other hand, it is obvious that if we replace the formulas (3.21) - (3.24) (taking into account the expressions (3.8) - (3.12) too) in the right hand sides of the formulas (3.1) - (3.7) and (3.17) - (3.24) we will obtain relationships between stationary values

of all MEK/ERK pathway components and initial values of the slow varying concentrations. The last ones can be considered as a direct connection between input (initial values) and output (stationary values) pathway signals. In addition the main advantage of such connection is that it can be experimentally verified, i.e. it can be rejected or confirmed by experiments. In the last case we hope that these relationships could contribute the biologists to clarifying of the cancer disease mechanisms.

4. CONCLUSION

The investigation made in this paper is focused on analysis of the quasi-stationary dynamics of a MEK/ERK signal transduction process mediated by the protein RKIP. By solving of the quasi-stationary approximation of an original pathway model analytical relationships between stationary and initial values of all protein concentrations involved in the MEK/ERK cascade are determined. It is proved that the behaviour of the signal output determines only of initial slow varying pathway concentrations. Moreover a leading role of the initial values of RKIP protein complexes (RKIP/Raf-1 and ERKpp/RKIP/Raf-1) near to the pathway stationary state is theoretically established. That leads to the conclusion that near the quasi-steady-state the experimentally determined regulatory functions of the protein RKIP on the MEK/ERK pathway activity transmit to its conjugated forms.

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REFERENCES

1. Kolch W., Meaningful Relationships: The Regulation of Ras/Raf/MEK/ERK Pathway by Protein Interactions. *Biochem. J.*, 2000, 351, 289-305.
2. Yeung K., P. Janosch, B. McFerran, D.W. Rose, H. Mischak, J. M. Sedivy, W. Kolch, Mechanism of Suppression of Raf/MEK Extracellular Signal-regulated Kinase Pathway by the Raf Kinase Inhibitor Protein, *Mol. Cell Biol.*, 2000, 20, 3079-3085.
3. Yeung K., T. Seitz, S. Li, P. Janosch, B. McFerran, C. Kaiser, F. Fee, K. D. Katsanakis, H. Mischak, D. W. Rose, J. Sedivy,

W. Kolch, Suppression of Raf-1 Kinase Activity and MAP Kinase Pathway Signaling by an Inhibitor Protein RKIP, *Nature*, 1999, 401, 173-177.

4. Timmer J., T. G. Muller, I. Swameye, O. Sandra, U. Klingmuller, Modelling the Nonlinear Dynamics of Cellular Signal Transduction, *Int. J. Bifurcation & Chaos*, 2003, 14, 2069-2079.
5. Heinrich R., B. G. Nell, T. A. Rapport, Mathematical Models of Protein Kinase Signal Transduction, *Mol. Cell*, 2002, 9, 957-970.
6. Cho K-H., S-Y. Shin, H-W. Kim, O. Wolkenhauer, B. McFerran, W. Kolch, Mathematical Modeling of the influence of RKIP on the ERK Signaling Pathway, *CSMB*, 2003, 127-141.
7. Cho K-H., S.-Y. Shin, W. Kolch, F. Fee, P. Wellstead, O. Wolkenhauer, Systems Biological Investigations into the Positive Feedback Mechanism by RKIP in the Ras/Raf/MEK/ERK Signal Transduction Pathway, Technical report, 2004, College of Medicine and Korea Bio-MAX Institute, Seul National University, Korea.
8. Petrov V., E. Nikolova, O. Wolkenhauer, Driver Identification of the Ras/Raf/MEK/ERK Signal Transduction Pathway, *Comptes rendus de l'Academie bulgare des Sciences*, 2005, 58(6), 639-644.
9. Nikolova E., Reduction of Dimensionality of Ras/Raf/MEK/ERK Signal Pathway Dynamical Model, In Ivanov Y. (Ed.). *Proceedings of the 10 Jubilee Congress on Theoretical and Applied Mechanics*, II, Varna, Bulgaria,13-16 September 2005, 170-176.
10. Petrov V., E. Nikolova, O. Wolkenhauer, Reduction of Nonlinear Dynamic Systems with an Application to Signal Transduction Pathways, *IET Syst .Biology*, 2007, 1(1), 2-9.
11. Nikolova E., New Result in Ras/Raf/MEK/ERK Signal Pathway Dinamical Model, *Comptes rendus de l'Academie bulgare des Sciences*, 2006, 59(2), 143-150.
12. Tichonov A. N., Systemy Differentsialnyh Uravneniy, Soderjashchie Malye Parametry pri Proizvodnyh, *Math. Sbornic*, 1952, 31, 575-586. (in Russian)