MAPK Module: Biological Basis, Structure, Mathematical Model and Dynamical Analyse

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***Abstract*— In this paper we present mitogen-activated protein kinase (MAPK) module: its biological definition, structure, and model. In modelling stage, we build on result of [9], and we include newly experimentally observed processes to capture more on real dynamic of cell: cross-linking among the different modules of MAPK and/or cross-linking with other pathways; influence of Phosphatase's, and influence of phosphorylated kinase kinase (KKP) found to have profound effect on module dynamics. For the chosen set of experimentally verifiable parameters we perform dynamic analyze. In investigation of bifurcation, we find *Hopf Bifurcation* as the only type of bifurcation observed.**

I. Introduction

The mathematical modelling of biological processes has become crucial for future developments in biological sciences and medicine [10]. Specifically, mathematical models enable computer simulations of biochemical signaling networks that provide i) quantitative predictions about their behavior, ii) an understanding of the role of specific biological modules within complex signaling pathways, and ultimately, iii) information for disease treatment and drug discovery [8].

This paper is devoted to the dynamically analysis of an important class of biological processes called the mitogen-activated protein kinase (MAPK) cascade. MAPK cascades are present in a variety of intracellular signaling pathways that control cellular functions such as cell growth, cell differentiation, apoptosis, cancer, and organ development. MAPK cascades are ubiquitous in eukaryotes, and their basic structure is well conserved from yeast cells to human cells [9]. In particular, the MAPK cascade is a family of serine/threonine (or tyrosine) kinases that relay signals from the cell membrane to targets in the cell cytoplasm and/or nucleus in response to a wide range of extracellular stimuli (e.g., osmotic pressure, heat/cold, drought, light, and hormones) [10]. Substantial research has been conducted on MAPK cascades that has contributed to the understanding of regulation of cell development and mechanisms by which cells respond to external signals. In fact, kinases in general are currently the second most popular drug target class in the pharmaceutical and biotech industries [4].

The dynamic analysis of MAPK cascades [9], [6], [11] have discovered that MAPK cascades possess different, interesting dynamic behaviors depending on the structure of the cascade and the value of certain kinetic parameters. The multi-level nature of the MAPK cascade provides signal amplification and consequently ultrasensitive (sigmoidal) input-output behavior, i.e., a small change in the stimulus can result in a large change in the response [6]. MAPK cascades can contain multiple positive and negative feedback loops. It was shown that negative feedback is responsible for the regulation not only of the species directly targeted by feedback, but also of signals downstream. In [9], it was shown that the combination of negative feedback and ultrasensitivity can cause sustained oscillations in MAPK cascades. Interestingly, the combination of positive feedback and ultrasensitivity leads to a bistable behavior as demonstrated in [11].

In this paper, we continue the study of the MAPK cascade dynamic behavior by building upon the models proposed in [6], [9]. In particular, we introduce the following features in the model of [9]. First, we take into account the action of phosphatases on the reverse reactions of the MAPKK and MAPK levels (see KKP'ase and KP'ase in Fig. 1) since it has been experimentally observed that phosphatases have profound influence on the cascade dynamics and regulation [6]. Second, we take into account the activation of K and KP at the third level by KKKP (see blue lines in Fig. 1), which has also been recently experimentally verified. Third, MAPK cascades are known to be interlinked with other signaling pathways. As such, we include activation and inhibition at the MAPKKK level due to *cross-talks* from other pathways that share the same components (see yellow and green lines in Fig. 1). The cross-talk inputs are included only at the first level since this is where they have the most influence on the whole cascade. Finally, we note that our model includes noncompetitive inhibition of KKK by KPP as in [9] (see the negative feedback, red line in Fig. 1).

The MAPK cascade consists of at least three levels — a MAPK kinase kinase (KKK), a MAPK kinase (KK), and a MAPK (K) — which are linked in various ways to upstream receptors and downstream targets. The activated kinase at each level phosphorylates the kinase at the next level down the cascade [6], [9], [10]. That is, KKKs activate KKs by phosphorylation at two conserved serine residues and KKs activate Ks by phosphorylation at two conserved serine/threonine and serine/tyrosine residues. On the other hand, the deactivation of the kinases is catalyzed by enzymes called phosphatases. See Fig. 1 for an illustration.

II. MAPK Biological modules

Discovery and Classification.

About 15 years ago, researchers studying the metabolic responses to mating pheromones by yeast discovered novel protein kinases that interfered with mating pheromone-induced growth arrest and mating-induced cell fusion. From

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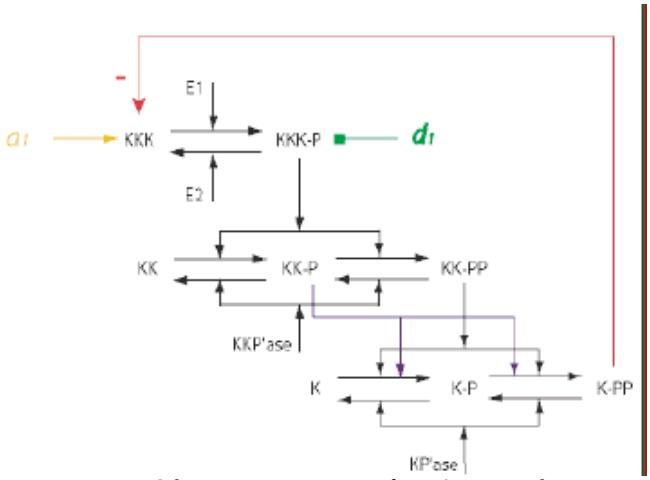

Fig. 1. Schematic representation of a MAPK cascade.

this beginnings, studies of MAPK superfamily have grown explosively.

Families of Kinases. *MAPK* kinases are classified according to substrate they react on: *Adenylate kinase*, *Creatine kinase*, *Pyruvate kinase*, *hexokinase*, *Nucleotide diphosphate kinase*, *Thymidine kinase*, and *Protein kinases*, making its largest group. They modify (by covalently attaching phosphate group to one of three amino acids that have a free hydroxyl group: Ser, Thr, Tyr) activity of target proteins, which are used to transmit signals and control processes in cells. Phosphorylation of the MAPK results in conformational change and a more then 1000-fold increase in specific activity. This results to functional change of the target protein (substrate), by changing its activity, cellular location or association with other proteins. Up to 30% of all proteins may be modified by kinase activity, and kinases are known to regulate majority of cellular pathways, especially those involved in signal transduction. The human genome contains about 500 protein kinase genes, constituting 2% of all genes in eukaryotic.

Families of Protein Kinases. Among the protein kinases, there is a distinction to: *Serine/Threonine specific protein Kinases*, *Tyrosine specific protein Kinase*, *Histidine specific protein kinases*, *Asparatic acid/glutamic acid specific protein kinases*, according to amino acid(s) they act on. We will be concerned with the first two, i.e. Ser/Thr and Tyr specific protein kinases. In biochemical process, substrate to be phosphorylated aligns with the kinase by several key amino acids (usually through hydrophobic forces and ionic bonds), emphasizing specificity of kinase to substrate.

MAPK family. There are several, namely five different MAPK cascades determined: ERK, JNK, p38, ERK2, and recently discovered ERK5. The main differences are in stimulating factor and biological output.

ERK module, responds primarily to growth factors and mitogens and stimulates transcriptional responses in nucleus which include activators of transcription factors. Generally, activation of an ERK signaling pathway has a role in mediating cell division, migration and survival.

JNK module, responds to a variety of stress signals including heat shock, osmotic stress, proimflammatory cytokines, ischemia, and UV exposure. It is activated by dual phosphorylation at the *Thr-Pro-Tyr motif*. Targets are transcription factors like c-Jun, AP-1, p53, etc. that help to regulate gene expression in response to a variety of cellular stimuli. Activation of JNK signaling cascade generally results in apoptosis, and has important roles in tumorigenesis and inflammation.

p38 module, responds to environmental stresses, including heat, osmotic and oxidative stresses, ionizing radiation, as well as inflammatory cytokines and tumor necrosis factor (TNF) receptor signaling. It is activated by dual phosphorylation at the *Thr-Gly-Tyr motif*. Upstream kinases that act on p38 have preferential effects on different p38 isoforms: $\alpha, \beta, \gamma, \delta, -2$. α and β isoforms are responsible for the activation of heat shock proteins, γ and δ isoforms activate some transcription factors that include ATF2, Sap-1a, Stat1, etc. Generally, the p38 subfamily is involved in affecting cell motility, regulation of gene expression, transcription and chromatin remodeling.

Medical diseases connected. Disregulated kinase activity is a frequent cause of disease, particularly cancer, where kinases regulate many aspects that control cell growth, movement and death. MAPK is involved in ischemic injury, liver regeneration, cardiac hypertrophy, rheumatoid arthritis, immune response, learning and memory, cancer, osmotic responses, host-parasite interactions and apoptosis, among the others. Drugs which inhibit specific kinases are being developed to treat several diseases, like *Cleevec* and *Iressa*. Critics of this drugs is left for further research [7].

III. Modeling of the MAPK Cascade.

The modelling of the MAPK cascade consist of two parts: the biochemical modelling, where we develop the set of chemical reactions, and the mathematical modelling, where the set of chemical reactions are described as a set of ordinary differential equations (ODE's).

A. Biochemical Model

The set of biochemical reactions for the **first level** of the MAPK cascade shown in Fig. 1 is given by

$$\begin{aligned} KKK + E_1 &\rightleftharpoons KKK.E_1 \rightarrow KKKP + E_1 \\ KKKP + E_2 &\rightleftharpoons KKKP.E_2 \rightarrow KKK + E_2 \end{aligned}$$

Influence of *negative feedback* is modeled as *noncompetitive inhibition*, where KPP react with E_1 which becomes incapable any more to catalyze KKK:

$$KPP + E_1 \rightleftharpoons KPP.E_1$$

Influence of *positive feedback* is modeled on the following way (for the first level only):

$$KKK + KPP \rightleftharpoons KKK.KPP \rightarrow KKKP + KP$$

Influence of *cross-talk* with other MAPK module or signaling pathways, can be modeled through *activation* and *deactivation* of initial cascade level:

$$\begin{aligned} KKK + a &\rightleftharpoons KKK.a \rightarrow KKKP + a \\ KKKP + d &\rightleftharpoons KKKP.d \rightarrow KKK + d \end{aligned}$$

The set of biochemical reactions for the **second level** of the MAPK cascade is given by

$$\begin{aligned} KK + KKKP &\rightleftharpoons KK.KKKP \rightarrow KKP + KKK \\ KKP + KKKP &\rightleftharpoons KKP.KKKP \rightarrow KKPP + KKK \end{aligned}$$

Influence of *Phosphatases* can be modeled as

$$\begin{aligned} KKP + KKP\prime ase &\rightleftharpoons KKP.KKP'ase \\ cont. &\rightarrow KK + KKP'ase \\ KKPP + KKP\prime ase &\rightleftharpoons KKPP.KKP'ase \\ cont. &\rightarrow KKP + KKP'ase \end{aligned}$$

The set of biochemical reactions for the **third level** of the MAPK cascade is given by

$$\begin{aligned} K + KKPP &\rightleftharpoons K.KKPP \rightarrow KP + KKP \\ K + KKP &\rightleftharpoons K.KKP \rightarrow KP + KK \end{aligned}$$

Influence of *Phosphatases* can be modeled as

$$\begin{aligned} KP + KP\prime ase &\rightleftharpoons KP.KP'ase \rightarrow K + KP'ase \\ KPP + KP'ase &\rightleftharpoons KPP.KP'ase \rightarrow KP + KP'ase \end{aligned}$$

Influence of *KKP phosphorylation* on K can be modeled as

$$\begin{aligned} K + KKP &\rightleftharpoons K.KKP \rightarrow KP + KK \\ KP + KKP &\rightleftharpoons KP.KKP \rightarrow KPP + KK \end{aligned}$$

Also note that *conservation of species laws* hold as given in Table 1.

Comparing the model presented in [9] with our model, we have modeled negative and positive feedback, cross-talk with other signaling pathways and/or other MAPK module, influence of kinase kinase Phosphatase (KKP'ase) and kinase Phosphatase (KP'ase), and influence of activation of third via phosphorylating by once phosphorylated kinase kinase (KKP). Last two cases are recently shown to have profound influence on module dynamics.

Further model extensions. Note that it is realistic to assume that each level, not only the first one (activation of $[KKK]$), can be influenced by cross-talk of other pathway, either stimulating it (activation by a) or destimulating it (deactivation by d). In that case both a and d can be regulators from other pathways. Also note that is realistic to assume that double-phosphorylated kinase of both second and third level may negatively feedback to their original kinase, i.e. noncompetitively inhibit it, not only third level, $[KPP]$. It is left to answer the degree of influence on module dynamics. The same holds for positive feedbacks. Also, it is reasonable to ask can $[KP]$ activates $[KK]$, and further phosphorylates $[KKP]$, and $[KKK]$ as well, on the similar manner as $[KPP]$ activates $[K]$ and $[KP]$.

B. Mathematical Model

In order to mathematically describe set of biochemical reactions above, in companion with moiety conservation equations given in Table 1, it is the basic approach to use Law of Mass Action and to trivially write down set of ordinary differential equations (ODE's), [1]. In order to obtain dynamic model from the set of biochemical reaction presented in previous section, let us define states of the system: $y_1 = [KKK]$, $y_2 = [KKKP]$, $y_3 = [KK]$, $y_4 = [KKP]$, $y_5 = [KKPP]$, $y_6 = [K]$, $y_7 = [KP]$, and $y_8 = [KPP]$.

Mathematical model of MAPK module is given by:

$$\begin{aligned} \dot{y}_1 &= v_2 - v_1 - v_a + v_d - v_p && (1) \\ \dot{y}_2 &= v_1 - v_2 + v_a - v_d + v_p && (2) \\ \dot{y}_3 &= v_6 - v_3 && (3) \\ \dot{y}_4 &= v_3 + v_5 - v_4 - v_6 && (4) \\ \dot{y}_5 &= v_4 - v_5 && (5) \\ \dot{y}_6 &= v_{10} - v_7 - v_{11} && (6) \\ \dot{y}_7 &= v_7 + v_9 - v_8 - v_{10} + v_{11} - v_{12} && (7) \\ \dot{y}_8 &= v_8 - v_9 + v_{12} && (8) \end{aligned}$$

where rates of reactions are given below.

$$\begin{aligned} v_1 &= \frac{V_{\max 1}\, x_1}{(1+(\frac{x_8}{K_I})^n)(K_{M1}+x_1)},\ V_{\max 1} = k_2[E_1]_T \\ v_2 &= \frac{V_{\max 2}\, x_2}{K_{M2}+x_2},\ V_{\max 2} = k_2[E_2]_T \\ v_3 &= \frac{k_3\, x_2\, x_3}{K_{M3}+x_3},\ v_4 = \frac{k_4\, x_2\, x_4}{K_{M4}+x_4} \\ v_5 &= \frac{V_{\max 5}\, x_5}{K_{M5}+x_5},\ V_{\max 5} = k_5[KKP'ase]_T \\ v_6 &= \frac{V_{\max 6}\, x_4}{K_{M6}+x_4},\ V_{\max 6} = k_6[KKP'ase]_T \\ v_7 &= \frac{k_7\, x_5\, x_6}{K_{M7}+x_6},\ v_8 = \frac{k_8\, x_5\, x_7}{K_{M8}+x_7} \\ v_9 &= \frac{V_{\max_9}\, x_8}{K_{M9}+x_8},\ V_{\max 9} = k_9[KP'ase]_T \\ v_{10} &= \frac{V_{\max_{10}}\, x_7}{K_{M10}+x_7},\ V_{\max 10} = k_{10}[KP'ase]_T \\ v_{11} &= \frac{k_{11}\, x_4\, x_6}{K_{M11}+x_6},\ v_{12} = \frac{k_{12}\, x_4\, x_7}{K_{M12}+x_7} \\ v_a &= \frac{V_{\max a}\, x_1}{K_{Ma}+x_1},\ v_d = \frac{V_{\max d}\, x_2}{K_{Md}+x_2},\ v_p = \frac{k_{p1}\, x_8\, x_1}{K_{Mp}+x_1} \end{aligned}$$

Initial conditions and parameter values are given in Table 2 and Table 3, respectively.

IV. Physiological Control of MAPK Module

Because *kinases* have profound effect on a cell, their *activity is highly regulated*. Kinases are turned on or off by phosphorylation (as described in model above), by binding of activator

Table 1 Conservation of Species Equations
$[KKK]_T$= [KKK]+[KKKP]+[KKK.E_1]+[KKKP.E_2]+[KKK.KPP]
$[[E_1]_T$= [E_1]+[KKK.E_1]
$[E_2]_T$= [E_2]+[KKKP.E_2]
$[KK]_T$= [KK]+[KKP]+[KKPP] +[KK.KKKP]+[KKP.KKKP]
+[KKP.KKP'ase]+[KKPP.KKP'ase]
$[K]_T$= [K]+[KP]+[KPP]+[K.KKP] +[K.KKPP]+[KP.KKP]
+[KP.KKPP]+[KP.KP'ase]+[KPP.KP'ase]
$[KP'ase]_T$= [KP'ase]+[KP.KP'ase]+[KPP.KP'ase]
$[KKP'ase]_T$= [KKP'ase]+[KKP.KKP'ase]+[KKPP.KKP'ase]

proteins or inhibitor proteins (modeled through activation and deactivation, see Fig. 1), or small molecules, or by controlling their location in the cell relative to their substrates. MAPK signalling pathways are susceptible to regulatory inputs at multiple levels within the cascade, as well as via multiple mechanism. Physiological control of MAPK can include cell/tissue specific expression patterns for each MAPK module, specificity of the stimuli that can trigger each MAPK module, specificity of substrates that are targets of each MAPK in each cell type, modification MAPK responses via activators, inhibitors, scaffolding proteins, sequential interactions with other proteins in a cascade, positive and negative feedback loops, and cross-talk among MAPK and other signaling pathways. In brief,

Specificity is in majority of cases solved since enzymes are substrate specific. Way to achieve specificity of a pathway is via the type of MAPKKK activated by a signal. **Scaffold Proteins** make large complexes with kinases at all three levels, regulating passing of signals on downstream kinases, therefore maintaining specificity of the response upon the stimulus. Upon stimulus **Adapter Proteins** adapter proteins makes a complexes that further recruits enzymes that are responsible for cascade activation. For example, JNK module is regulated by adapter proteins taking signals from TNF receptors. Next, MAPK activity is **regulated by subcellular location**: ERK, during mitosis, are situated at the kinetochores, but during M phase on the mitotic apparatus. JNK can be found along microtubules.

Regulation via stimulus intensity. For specific biological responses, the timing and duration of the stimulus also has a direct impact on the type of response that cells make to a signal as well as the cell type affected. Thus, sustained or transient signals through ERK, for example, will determine whether a cell's response is differentiation or proliferation. Influence of stimulus input has been modeled in this paper (bifurcation w.r.t. V_1, i.e. E_1).

Regulation by Phosphatases is key control mechanism in MAPK module, by dephosphorylation of activated kinases. In case of JNK module major influence is achieved by MKP phosphatase. Their influence has been modeled in this paper ($KP\prime ase$). The activities of MKPs are also defined by their subcellular location. MKP3 is known to be cytoplasmic, whereas MKP1 is only found in the nucleus.

Table 2
Initial Conditions
[nM]
[KKK]=100
[KK]=300
[K]=300
[KKKP]=0
[KKP]=0
[KP]=0
[KKPP]=0
[KPP]=0

Table 3	
Parameter Values	
V[nMs^{-1}], k[s^{-1}], K[nM]	
V_{max1}=0.25	K_{M1}=10
n=1	K_I=9
V_{max2}=0.25	K_{M2}=8
k_3=0.025	K_{M3}=15
k_4=0.025	K_{M4}=15
k_5=0.75	K_{M5}=15
k_6=0.75	K_{M6}=15
k_7=0.025	K_{M7}=15
k_8=0.025	K_{M8}=15
k_9=0.5	K_{M9}=15
k_{10}=0.5	K_{M10}=15
k_{a1}=0.25	K_{Ma}=10
k_{d1}=0.25	K_{Md}=10

Communication between MAPK modules. Several of MAPK modules can run in parallel and there is a considerable degree of cross-talk between them, which creates multiple opportunities for modulating or fine-tuning responses to different signals. Specificity of the MAPK signaling pathways is greatest at the level of specific MKK activation of individual MAPKs, where there is the least amount of cross-talk. The MAPKKKs are also involved in cross-talk. In this paper we have modeled possible influence of communication on this first level.

Influence on other pathways, Cross-linking. MAPK cascades can also influence other signaling pathways and vice versa; there are several known examples of MAPK influences on outside signaling pathways. For example, ERKs can activate the JAK-STAT pathways. MAPK signaling cascades are influenced by other signaling pathways, including those linked with cAMP and calcium. In this paper we have modeled possible influence of other signalling pathways on first level.

Signal dependence. Obviously, there is potential for a considerable level of communication between the interlinked modules, the type and amount of which can vary widely depending upon factors, including the length, intensity and timing of signal, type of cell, and cell-specific receptor distribution at the plasma membrane.

V. Dynamic Analyze of MAPK Module: Numerical Results

A. Stability analysis.

In order to perform stability analyze of dynamic system (1), note that we have eight dimensional nonlinear system. Taking into account conservation of species equations, we can reduce the dimension of system to five. Determining the equilibria for 8D system, linearizing the system, calculating the Jacobian of system at specific equilibria, and calculating the eigenvalues, we will see that our system posses three zero eigenvalues. Implementing three conservation equations, and substituting $[KKKP]$, $[KKP]$, and $[KP]$ through other variables and constants (see Table 1), we end up with 5D system which is much easier to handle.

For the particular set of parameters as given in Table 2 and Table 3, system (1) has *equilibria* at: $[KKK] = 81.938; [KK] = 272.22; [KKPP] = 7.8503; [K] = 288.5; [KPP] = 2.5738$. Further, system has two complex eigenvalues with negative real part, and three negative real eigenvalues, what classifies the system (for chosen set of parameters) as *stable* (determined in XPPAUTO). Actually, oscillatory behaviour due to complex eigenvalues will eventually disappear, showing type of **transient behaviour**. This type of behaviour causes cell proliferation as possible biological outcome. For example, if we change parameter V_1 to 5 (any value in range $\{0.3842, 6.248\}$ for our system, initially it is 0.25) response will give **sustained oscillations** what classifies system as marginally stable, i.e. unstable approaching *periodic solution*. Response can be trivially obtained in MatLab environment. This type of behaviour causes cell differentiation as possible biological outcome.

We can think of parameter V_1 as bifurcation parameter. For smaller values of parameter V_1 (precisely, less then 0.3842), response gives *stable equilibria*. When parameter attain value V_1=0.3842 *Hopf Bifurcation* occur yielding as a consequence *periodic solution*. Increasing the parameter V_1 value further, second point of Hopf bifurcation is $V_1 = 6.248$ is attained, after which system again rich stable equilibrium points. Response is omitted due to space limitations. Obtained responses correspond to one obtained in bifurcation study by using XPPAUTO (given in Fig. 7).

Next, we have modelled **influence of once phosphorylated kinase on second level (KKP)** that can activate kinases at third level: kinase (K) and once phosphorylated kinase (KP). Influence can be tracked by usual simulation through varied parameters k_{11} and k_{12}. Responeses are given in [7].

Response of added **cross-linking due to activation/deactivation** of system by other signaling pathway and/or other MAPK module can be seen through incorporation of activation v_a and deactivation v_b rates in system (1), respectively. In case of deactivation, for purpose of richer graphical representation, we show response of system (1) with deactivation included, and in case of Hill's coefficient being $n = 10$ (initially, $n = 1$), and varying deactivation signal, through k_d (similarly, signal D). Responses are given at Fig. 2.

B. Bifurcation Analysis.

The only bifurcation type of system (1) is *Hopf bifurcation*. With respect to changes of several parameters, namely V_1, n, V_i, etc., our system can undergo *Hopf bifurcation* from *stable fixed equilibria* to *stable periodic solution*, so bifurcation analysis enabled us to trace changes of the dynamics of the systems. Bifurcation software used is XPPAUTO. For more powerful and interactive environment reader is advised to use UNIX based AUTO program.

Calculations and analytical results show existence of *Hopf Bifurcation [5]* for system (1), as only observed type of bifurcation. We are primary concerned here with numerical results as they show both type of bifurcation at exact values

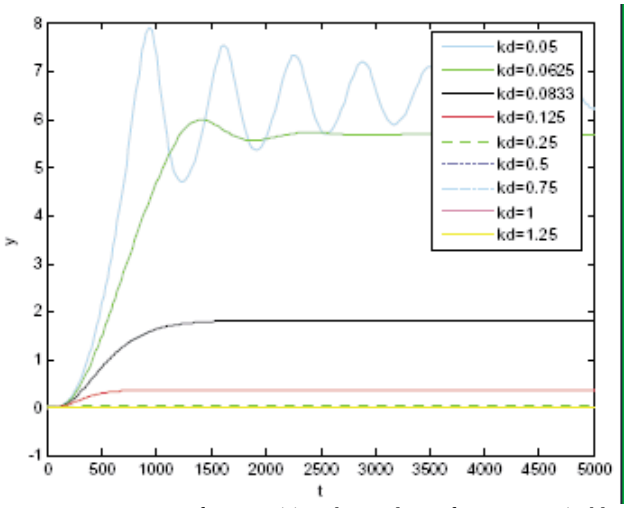

Fig. 2. Time response of system (1) with initial set of parameters (Table 2 and 3) but n=10, and deactivation v_d included. As parameter k_d (similarly, deactivation signal D) is varied, response pass from transient behaviour to stable equilibria.

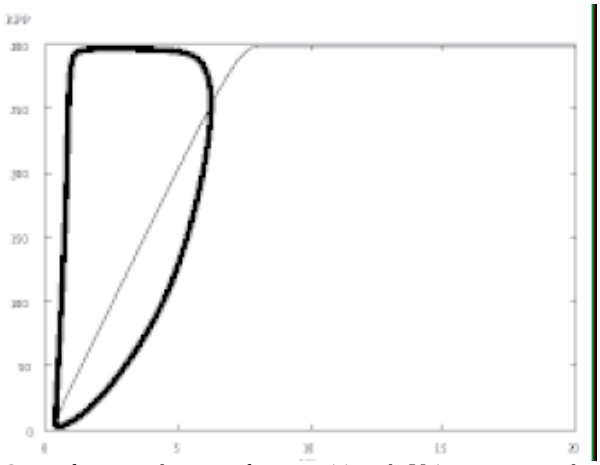

Fig. 3. Bifurcation diagram of system (1) with V_1(i.e. input signal E1) as bifurcating parameter. At values V1 below 0.3842, *stable equilibria* is reached, at V1 values within range {0.3842, 6.248} *periodis solution* occurs, and at interval point system pass through *Hopf bifurcation*.

of parameters where particular bifurcation occurs. We show bifurcation diagram for system (1) when we vary V_1 (i.e. input signal E_1), *Hill's coefficient* and similarly results can be obtained for changes of n, and integration constant V_i, as parameter representing influence of negative feedback.

Stable equilibrium point at specific value of V_1 for chosen set of parameters, and considering only one bifurcation parameter, pass through *Hopf Bifurcation* yielding *periodic orbit* (steady state). For the particular V_1 parameter bifurcation points are at $V_1 = 0.3842$ and $V_1 = 6.248$, i.e. within the range $\{0.3842, 6.248\}$ periodic solution appear, and system tends to stable equilibrium point. Period of oscillations of periodic orbit are $T = 637.7$ and $T = 1227$, respectively. Bifurcation diagram is given at Fig. 3.

Similarly, bifurcation diagram for system (1) w.r.t. changes of Hill/s coefficient is obtained. Bifurcation occur at value $n = 2.809$, with period of oscillating $T = 560.6$, where solution passes from *stable equilibria* to *periodic orbit*, [7].

Further, bifurcation diagram for system (1) w.r.t. changes of integration constant V_i, are obtained which interprets strength of negative feedback. Bifurcation occur at value $V_i = 277.8$, with period of oscillating $T = 1409$, where solution passes from *stable equilibria* to *periodic orbit*. Second point of *Hopf bifurcation* appears at $V_i = 649.7$, with period of oscillating $T = 1867$, [7].

Next, we perform **bifurcation analysis with cross-linking via activation** from other signaling pathway and/or MAPK module included. We have produced bifurcation diagrams as in case of system (1), and obtained bifurcation diagrams for changes of V_1, n, and V_i, as bifurcation parameters. Results are as diagram shown on Fig. 7 and are omitted here. Numerical values — with the set of parameters given in Table 2 and Table 3, varying V_1 as bifurcation parameter, we have slight change of bifurcation values where *Hopf Bifurcation* appear at $V_1 = 0.3633$ (period 666.3), and $V_1 = 5.492$ (period 1269). The values of V_1's are slightly lower then in initial case, what is a direct consequence of rate v_a included in (1), and is easily understood. Periods are slightly increased. Bifurcation diagram have appearance as Fig. 7, [7].

Further, we modelled **influence of changing** concentration of **kinase kinase Phosphatase KKP'ase,** i.e. taking it as bifurcation parameter. For system (1), with set of parameters given in Table 2 and Table 3, but $[KKP\prime ase] = 0.5$, system have two complex eigenvalues with negative real parts, and three negative real eigenvalues. Equilibrium points for that values are: $[KK] = 78.568, [KK] = 254.93, [KKPP] = 13.957, [K] = 290.59, [KPP] = 1.964$. Response is given in [7] . Increasing the $[KKP\prime ase]$ concentration biologically can change fate of cell from cell differentiation to cell proliferation.

For the system (1), with set of parameters given in Table 2 and Table 3, but $[KKP\prime ase] = 0.5$, bifurcation values for *parameter* V_1 for example, will slightly drop to $V_1 = 0.3865$(period 602.3), and $V_1 = 6.441$ (period 1168). Appearance is the same as in Fig. 7, [7].

Further, we modelled **influence of changing** concentration of **kinase Phosphatase KP'ase,** i.e. taking it **as bifurcation parameter.** For purpose of numerical exposition, for system (1), with set of parameters given in Table 2 and Table 3, but $[KP\prime ase] = 1.5$, system have two complex eigenvalues with negative real parts, and three negative real eigenvalues. Equilibrium points for that values are: $[KKK] = 69.371, [KK] = 280.97, [KKPP] = 4.9304, [K] = 294.48, [KPP] = 0.92065$. Bifurcation diagram has appearance as in Fig. 7, [7]. Increasing the KKP'ase concentration biologically can change fate of cell from cell differentiation to cell proliferation.

VI. Conclusions and Future Work

In this paper we presented basic biological basis and classification of MAPK Module and its structure. We have performed modelling: biochemical and mathematical, to capture more reality within cell. To achieve this we have incorporated the negative (similarly, positive) feedback, we have added activation (similarly, deactivation) by cross-linking with other MAPK module and/or pathway. Next, we have modelled influences of phosphatases (KKP'ase and KP'ase, that reacts on second and third level, respectively), and once phosphorylated kinase kinase (KKP) on second level that is capable to activate (i.e. once phosphorylate) MAPK on third level. The last two show considerable influence on dynamics of MAPK module, specifically on amplitude, duration and type of output signal KPP (double phosphorylated kinase) that will further activate other kinase or transcription factor with certain biological function, like cell death or survival. Numerical results of derived mathematical model are performed with respect to experimentally verifiable set of parameters and corresponding initial concentrations. Time response, stability type, and numerical bifurcation analyze is performed in order to gather insight into structure, its reasons, and behaviour of MAPK module under variety of input stimuli and chosen parameters. Complete analytical study is under preparation, [7].

Future work will incorporate information from new experimental data to capture more reality of cell. At the same time, problem will be set in control framework to be easily tracked by mathematicians and control engineers. Theoretically, further work will be concentrated on Lyapunov function construction for MAPK module, dissipativity theory development and application, robustness and sensitivity results. It is of further interest to examine influence of noise that will lead to stochastical system representation. Of vital importance is then analyze of possible control mechanism, that will ultimately lead to drug design, via natural substances and tea.

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