2011 50th IEEE Conference on Decision and Control and
European Control Conference (CDC-ECC)
Orlando, FL, USA, December 12-15, 2011

Multistability and robustness of the MAPK pathway

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***Abstract*— In this paper, we propose a qualitative model of the MAPK signaling cascade and analyze its multi-stability properties. Building on our previous work, we employ Lyapunov and invariant sets theory to analyze the system. In particular we focus on the first stage of a class of MAPK cascades, known as the Mos subsystem, which is the target of a positive feedback loop. Under general assumptions, we show that the system is bistable when three equilibria are present, regardless of the specific feedback dynamics.**

I. Introduction

All living organisms rely on complex molecular circuitry to sense and react to their environment. To guarantee survival, these molecular networks should respond consistently to external stimuli, despite the variability of their components in every individual. A large body of research has highlighted that robustness to uncertainty is often a structural property of specific biological systems. However, there are few systematic methods to mathematically model and describe structural robustness. With a few exceptions, numerical studies are often the preferred approach to this type of investigation.

Analytical approaches to the study of robustness have been proposed in specific contexts. A series of recent papers [1], [2] focused on input/output robustness of ODE models for phosphorylation cascades. In particular, the theory of chemical reaction networks is used in [2] as a powerful tool to demonstrate the property of absolute concentration robustness. Indeed, the so–called deficiency theorems [3] are to date some of the most general results to establish robust stability of a chemical reaction network. Monotonicity is also a structural property that is useful to demonstrate robust dynamic behaviors of a class of biological models [4], [5]. Robustness has also been investigated in the context of compartmental models, which are often encountered in biology and chemistry [6].

In this paper, we focus on the robust stability of equilibria for a class of ordinary differential equation models describing the mitogen-activated protein kinase (MAPK) pathway. We follow a qualitative modeling framework that captures trends, positivity and boundedness of the interactions among the pathway components [7]. Exact, parametric mathematical expressions are not employed. To analyze the properties of these qualitative models, we use Lyapunov and invariant-sets methodologies.

Several experimental studies have highlighted the presence of feedback loops in the MAPK pathway, which result in different dynamic behaviors. We consider a specific positive-feedback topology that is described in detail in Section III. Such positive feedback has been extensively studied in the literature, since the biochemical analysis of Huang and Ferrell [8], [9] on the MAPK cascade in *Xenopus* oocytes. Depending on the dynamic properties assumed for the positive feedback loop, we prove that the system structurally exhibits a different number of equilibria and stability properties. However, we demonstrate that when the system presents three equilibria, bi-stability is guaranteed.

The MAPK cascade model properties that can be derived from our analysis are structurally robust because they are not inferred from specific mathematical expressions chosen to fit data. A limit of our theoretical investigation is that its systematic application to more detailed and higher order models is challenging. However, the set of techniques we employ can be successfully used to study a large class of simple systems, and are in general suitable for the analysis of structural robustness of biological networks, complementary to simulations and experiments.

The paper is organized as follows. In Section II we summarize our general approach [7] and provide useful definitions and background. In Section III, we propose a model for the considered MAPK pathway and state our main results. Finally, the proofs of our results are outlined in Section IV.

II. A general setup to investigate structural robustness

Consider a class of biological dynamical systems which are successfully modeled with ODEs and can be written as:

$$\dot{x} = f(x, u), \qquad x(0) = x_0, \tag{1}$$

where x is the system state, u models external inputs, and both are vectors of appropriate dimensions.

Robustness in our framework may be formally defined as follows.

Definition 1: Let $\mathcal{C}$ be a class of systems and $\mathcal{P}$ be a property pertaining such a class. Given a family $\mathcal{F} \subset \mathcal{C}$ we say that $\mathcal{P}$ is robustly verified by $\mathcal{F}$, in short robust, if it is satisfied by each element of $\mathcal{F}$.

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Countless examples can be brought about families $\mathcal{F}$ and candidate properties. In this work, we will focus on the property of stability, which is an important feature for the equilibria of biological networks [10], [5], [11].
We will consider a class of biological network models consisting of n first order differential equations

$$\dot{x}_i(t) = \sum_{j\in\mathcal{A}_i} a_{ij}(x_i,x_j)x_j - \sum_{h\in\mathcal{B}_i} b_{ih}(x_i,x_h)x_h + \\ + \sum_{s\in\mathcal{C}_i} c_{is}(x_s) + \sum_{l\in\mathcal{D}_i} d_{il}(x_l),\ i=1,2,\dots,n. \quad (2)$$

where x_i, $i = 1, ..., n$ are the dynamic variables. For the sake of notation simplicity, we are not denoting external inputs with a different symbol. Inputs can be easily included as dynamic variables $\dot{x}_u = w_u(x_u, t)$ which are not affected by other states and have the desired dynamics. The sets $\mathcal{A}_i$, $\mathcal{B}_i$, $\mathcal{C}_i$, $\mathcal{D}_i$ denote the subsets of variables affecting x_i.

The states of equation (2) are associated to concentrations of biochemical species. The different mathematical terms are in turn associated with specific biological processes. The terms $a_{ij}(x_i, x_j)x_j$ are associated with production of species; these functions are *possibly unbouded* terms. Similarly, terms $b_{ih}(x_i, x_h)x_h$ represent degradation or conversion of species, and are possibly unbounded terms. Finally, $c(\cdot)$ and $d(\cdot)$ are associated with catalytic or cooperative production or degradation and are monotonic, nonlinear, *necessarily bounded* terms. (In the literature, these processes are typically modeled with Michaelis-Menten or Hill functions [12].)
We assume that system (2) satisfies the following assumptions:

Assumption 1: (Smoothness) The involved functions a_{ij} and b_{ih} c_{is} and d_{il} are unknown, nonnegative and continuously differentiable.

Assumption 2: Functions $f_{ih}(x_i, x_h) := a_{ih}(x_i, x_h)x_h$ and $g_{ih}(x_i, x_h) := b_{ih}(x_i, x_h)x_h$, are strictly increasing in x_j and x_h respectively:

$$\frac{\partial f_{ij}(x_i,x_j)}{\partial x_j} > 0, \qquad \frac{\partial g_{ih}(x_i,x_h)}{\partial x_h} > 0, \quad \forall x.$$

Assumption 3: (Saturation) Functions $c_{is}(x_s)$ and $d_{il}(x_l)$ are nonnegative and, respectively, non–decreasing and non–increasing. Moreover $c_{is}(\infty) > 0$ and $d_{il}(0) > 0$.

Assumption 4: Functions $b_{ih}(x_i, x_h)$ are null at the lower saturation levels: if $x_i \neq x_h$ then $b_{ih}(0, x_h) = 0$, $\forall x_h$.

Remark 1: Note that the "particular form" chosen for the terms $a_{ij}(0, x_j)x_j$ and $b_{ih}(0, x_h)x_h$ is the natural generalization of the linear case $a_{ij}x_j$ and $b_{ih}x_h$ often adopted in approximated models. Note that any smooth function (then with bounded derivative) of the form $f_{ij}(x_i, x_j)$ such that $f_{ij}(x_i, 0) = 0$ (or $g_{ih}(x_i, 0) = 0$), $\forall x_i$, and strictly increasing in the second argument can be written as $f_{ij}(x_i, x_j) = a(x_i, x_j)x_j$ (or $g_{ih}(x_i, x_h) = b(x_i, x_h)x_h$).
The general model (2) is a nonlinear positive system, and its investigation will be restricted to the positive orthant. For brevity, we have considered functions depending on two variables. Clearly, model (2) can be easily generalized to include terms as $a(x_i, x_j, x_k, \dots)$, $b(x_i, x_j, x_k, \dots)$, $c(x_i, x_j, x_k, \dots)$, $d(x_i, x_j, x_k, \dots)$. with suitably extended assumptions A1-A4 to multivariate functional terms.

The construction of a dynamic model from trend, sign and boundedness features of the interactions among the system variables can be aided by constructing a graph [7]. The nodes of the graph are the system's species, and the qualitative relationships between the species are associated with four types of arcs as shown in Fig. 1. For simple networks, this type of graph may provide intuition regarding their behavior and may facilitate their structural robustness analysis. (A systematic theoretical analysis of these graphs is a topic of current and future work and is not addressed in this paper.)

Fig. 1. The a, b, c and d types of arcs.

A. Non–smooth Lyapunov functions

Non–smooth Lyapunov functions are particularly useful to analyze the class of models (2), [7]. For the reader's convenience, here we briefly recall their main features. Consider the convex function:

$$V(x-\bar{x}) = \max_i V_i(x-\bar{x}),\ i=1,...,N,$$

where each $V_i(\cdot)$ is smooth and convex, and we assume that $V(\cdot)$ is positive definite. The set of active functions is never empty and is defined as: $\mathcal{A} = \{i : V_i(x-\bar{x}) = V\}$. If we define the generalized Lyapunov derivative as:

$$D^+V(x-\bar{x}) \triangleq \max_{i\in\mathcal{A}} \nabla V_i(x-\bar{x})f(x),$$

then the condition for stability becomes:

$$D^+V(x-\bar{x}) < \kappa(x-\bar{x}), \quad \kappa(\cdot) \text{ negative definite.}$$

We refer the reader to [13] for further details; more recent works on this topic are [14] and [15].

B. Metzler matrices

As previously mentioned, the general model in equation (2) is a positive nonlinear system. It is useful to recall that positive affine systems are a special case of monotone systems. Positive affine systems are in general defined by a model $\dot{x}(t) = Ax(t) + b$, where b is a nonnegative vector and A is is a Metzler matrix (M–matrix), namely, $a_{ij} \geq 0$ if $i \neq j$. Metzler matrices are a recurring element in the analysis of the models considered in this paper. These matrices exhibit a real dominant eigenvalue, and the following property holds.

Proposition 1: The following statements are equivalent for an M–matrix: (1) A is stable (i.e. have negative real part eigenvalues); (2) A has no nonnegative real eigenvalues; (3) The characteristic polynomial $p(s) = det(sI - A)$ has positive coefficients.

III. The MAPK signaling pathway

Mitogen-activated protein (MAP) kinases are proteins that respond to the binding of growth factors to cell surface receptors. The pathway consists of three enzymes, MAP kinase, MAP kinase kinase (MAP2K) and MAP kinase kinase kinase (MAP3K) that are activated in series. By activation or phosphorylation, we mean the addition of a phosphate group to the target protein. Extracellular signals can activate MAP3K, which in turn phosphorylates MAP2K at two different sites; in the last round, MAP2K phosphorylates MAPK at two different sites. The MAP kinase signaling cascade can transduce a variety of growth factor signals, and has been evolutionary conserved from yeast to mammals.

Several experimental studies have highlighted the presence of feedback loops in this pathway, which result in different dynamic properties. Here we consider the well-known positive-feedback topology found in *Xenopus* oocytes [8], [9]. In this type of cells, Mos (MAP3K) can activate MEK (MAP2K) through phosphorylation of two residues (converting unphosphorylated MEK to monophosphorylated MEK-P and then bisphosphorylated MEK-PP). Active MEK then phosphorylates p42 (MAPK) at two residues. Active p42 can then promote Mos synthesis, completing the closed positive-feedback loop. From now on we will denote each species in the network as reported in Table I.

Let us now derive a qualitative dynamical model of the MAPK pathway, using the graph in Fig. 2 as a support. We assume that MAP1K, associated to the node labeled as node 1, is produced and degraded at some rate. Based on the four interaction categories previously introduced, we can associate the production of MAP1K to a positive and bounded activation function of type c_{10}; degradation can be plausibly associated to a function $b_{11}(x_1)x_1$. We assume that each MAP(i)K, for $i = 2, 3$, mediates the phosphorylation of MAP($i-1$)K through a Hill-type activation process, transforming it into a MAP($i-1$)K-P, phosphorylated at a single site; additionally, MAP(i)K mediates the addition of a second phosphate group, transforming MAP($i-1$)K-P into MAP($i-1$)K-PP. Consider the nodes of the graph at Fig. 2 associated to x_1, x_2 and x_3: it is natural to connect nodes x_1 and x_3 with a function of type $a_{13}(x_2)x_3$. Similarly for x_1 and x_4, and for activation of x_6, x_7 by x_4. Due to mass conservation, if MAP(i)K causes the increase of MAP($i-1$)K-P and MAP($i-1$)K-PP, then MAP(i)K also causes a decrease of MAP($i-1$)K and MAP($i-1$)K-P: this effect is taken into account by the arcs b_{21}, b_{31}, b_{54} and b_{64} in Fig. 2. Furthermore, one can assume a spontaneous loss of phosphate groups: MAP(i)K-P and MAP(i)K-PP decay into MAP(i)K and MAP(i)K-P respectively (mass conservation still holds). This bounded decay effect is taken into account, together with other kinase degradation processes, by arcs b_{33}, b_{44}, b_{66} and b_{77}; the simple loss of a phosphate group causes a bounded increase of the concentration of MAP(i)K and MAP(i)K-P, taken into account through the arcs c_{23}, c_{34}, c_{56} and c_{67}. Here, we neglect the input-mediated phosphorylation dynamics of the MAP3K protein [5]. Finally, we model the positive feedback between fully active p42 and Mos as the arc $\mu a_{17}(x_1)$, where μ is introduced for convenience as a feedback scaling term. Using the graph in Fig. 2 as a support, a dynamic model can be written as follows:

$$\begin{aligned}
\dot{x}_1 &= a_{17}(x_1)\,\mu x_7 + c_{10} - b_{11}(x_1)x_1 \\
\dot{x}_2 &= c_{23}(x_3) - b_{21}(x_2)x_1 \\
\dot{x}_3 &= a_{31}(x_2)x_1 + c_{34}(x_4) - b_{31}(x_3)x_1 - b_{33}(x_3)x_3 \\
\dot{x}_4 &= a_{41}(x_3)x_1 - b_{44}(x_4)x_4 \\
\dot{x}_5 &= c_{56}(x_6) - b_{54}(x_5)x_4 \\
\dot{x}_6 &= a_{64}(x_5)x_4 + c_{67}(x_7) - b_{64}(x_6)x_4 - b_{66}(x_6)x_6 \\
\dot{x}_7 &= a_{74}(x_6)x_4 - b_{77}(x_7)x_7.
\end{aligned} \tag{3}$$

The presence of the positive feedback in the MAPK cascade has been linked to a bistable behavior: the switch between two stable equilibria in *Xenopus* oocytes denotes the transition between the immature and mature state.

TABLE I

Definition of the state variables for the MAPK network

x_1	x_2	x_3	
MAP3K/Mos	MAP2K/MEK	MAP2K-P/MEK-P	
x_4	x_5	x_6	x_7
MAP2K-PP/MEK-PP	MAPK/p42	MAP1K-P/p42	MAP1K-PP/p42

Bi-stability may occur due to other phenomena, such as multisite phosphorylation [16], rather than due to feedback loops. However, a large body of literature focuses on bi-stability induced by the positive-feedback in the Huang-Ferrel model in *Xenopus* [17], [18]. In [8] the feedback $a_{17}(x_1)x_7$ was characterized, through *in vitro* studies, as a Hill-function with high cooperativity. In [5] instead, $a_{17}(x_1)x_7$ was assumed to be a first order linear term in the concentration of x_7. In Proposition 2 and following, we will explore the effects of different qualitative functional assumptions on the feedback loop dynamics $a_{17}(x_1)x_7$. We will highlight that the system loses its well-known bi-stability not only in the absence of feedback, but also when the feedback becomes unbounded. An unbounded positive feedback would be caused, for instance, by an autocatalytic process of MAP3K activation, mediated by active MAPK.

The term μx_7 introduces the positive feedback loop and represents a key parameter for the analysis to follow. Function $b_{11}(x_1)x_1$, functions $c_{23}(x_3)$, $b_{21}(x_2)$, $a_{41}(x_3)$ and $b_{44}(x_4)x_4$, and functions $c_{56}(x_6)$, $b_{54}(x_5)$, $a_{74}(x_6)$ and $b_{77}(x_7)x_7$ are increasing and asymptotically constant, i.e. they are bounded, their derivative is positive and decreasing. Moreover, $a_{31}(x_2) = b_{21}(x_2)$, $c_{34}(x_4) = b_{44}(x_4)x_4$, $b_{31}(x_3) = a_{41}(x_3)$, $b_{33}(x_3)x_3 = c_{23}(x_3)$ and $a_{64}(x_5) = b_{54}(x_5)$, $c_{67}(x_7) = b_{77}(x_7)x_7$, $b_{64}(x_6) = a_{74}(x_6)$, $b_{66}(x_6)x_6 = c_{56}(x_6)$. We assume c_{10} to be a positive constant.

Model (3) is consistent with the well-known dynamic model for MAPK pathway proposed in [5].
The graph in Fig. 2 can be partitioned considering three aggregates of variables, precisely $\{x_1\}$, $\Sigma_{234} = \{x_2, x_3, x_4\}$ and $\Sigma_{567} = \{x_5, x_6, x_7\}$. Signal x_1 is the only input for Σ_{234}, signal x_4 is the only input for Σ_{567}.

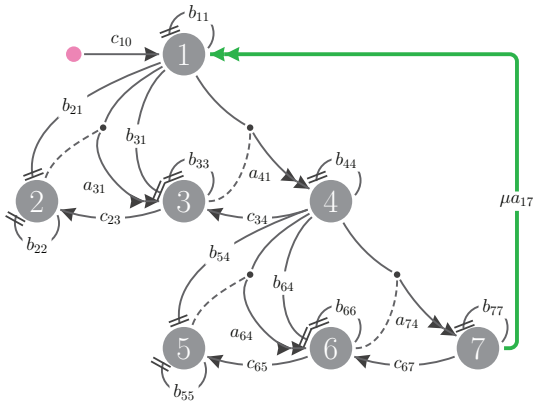

Fig. 2. Network corresponding to the MAPK pathway

Then x_7 is fed back to the first subsystems by arc a_{17}. Without the positive feedback loop, we will demonstrate that the system is a pure stable cascade. Note also that Σ_{234} and Σ_{567} can be reduced since $\dot{x}_2+\dot{x}_3+\dot{x}_4=0$ and $\dot{x}_5+\dot{x}_6+\dot{x}_7=0$ and therefore the following sums are constant:

$$x_2(t)+x_3(t)+x_4(t)=k, \qquad x_5(t)+x_6(t)+x_7(t)=h, \quad (4)$$

with $k \doteq x_2(0)+x_3(0)+x_4(0)$ and $h \doteq x_5(0)+x_6(0)+x_7(0)$. Since $x_i \geq 0$, all the variables but x_1 are bounded. The system can be studied by removing variables $x_3 = k - x_2 - x_4$ and $x_6 = h - x_5 - x_7$. We must assume that $c_{10} < \lim_{x_1\to\infty} b_{11}(x_1)x_1$, otherwise no equilibrium is possible.

The following propositions are our main results.

Proposition 2: For $\mu = 0$ the system admits a unique globally asymptotically stable equilibrium.

The next proposition concerns the existence of equilibria in the case in which an external positive feedback is present.

Proposition 3: For $\mu > 0$, the system may have multiple equilibria,[1] for specific choices of the involved functions a_{ij}, b_{ij}, c_{ij}. For $\mu > 0$ suitably large and $a_{17}(x_1)$ lower bounded by a positive number, then the system has no equilibria.

Proposition 4: For $\mu > 0$ suitably bounded and $a_{17}(x_1)$ increasing, or non-decreasing, and bounded, if multiple simple[2] equilibria exist, then such equilibria are alternatively stable and unstable. In the special case of three equilibria, then the system is bistable.
For $\mu > 0$ suitably bounded and $a_{17}(x_1)$ increasing asymptotically unbounded, then the number of equilibria is necessarily even (typically 0 or 2). Moreover, if we assume that there exists $\mu^* > 0$ such that the system admits two distinct equilibria for any $0 < \mu \leq \mu^*$, then one is stable, while the other is unstable.

Remark 2: The simplest case of constant a_{17} has been fully developed in [5] and [4], and it turns out that the system may exhibit bi-stability for suitable values of the feedback strength μ. Here we show that, for constant a_{17}, bi-stability is actually a robust property. Our results are consistent with the fact that the MAPK cascade is a monotone system and some of them could be demonstrated with the same tools used in [5], [4].
Note that the existence of an even number of equilibria in the presence of an unbounded a_{17}, is also consistent with previous results in the literature [19]. In this context, our contribution is that of inferring properties such as number of equilibria and mono or bi-stability starting from qualitative assumptions on the model, without invoking monotonicity or analytical solutions.

Remark 3: Finally, it is necessary to remark that our robustness analysis holds given model (3) and its structure. Other work in the literature shows that feedback loops are not required to achieve a bistable behavior in the MAPK cascade [16], when the dual phosphorylation and de-phosphorylation cycles are non-processive (i.e. sites can be phosphorylated/de-phosphorylation independently) and distributed (i.e. the enzyme responsible for phosphorylation/de-phosphorylation is competitively used in the two steps).

IV. Outline of the proofs

According to the mass conservation condition (4), we can model the MAPK network dynamics in the reduced form:

$$\begin{aligned}
\dot{x}_1 &= \mu\, a_{17}(x_1)x_7 + c_{10} - b_{11}(x_1)x_1,\\
\dot{x}_2 &= -b_{21}(x_2)x_1 + c_{23}(k - x_2 - x_4),\\
\dot{x}_4 &= a_{41}(k - x_2 - x_4)x_1 - b_{44}(x_4)x_4,\\
\dot{x}_5 &= -b_{54}(x_5)x_4 + c_{56}(h - x_5 - x_7),\\
\dot{x}_7 &= a_{74}(h - x_5 - x_7)x_4 - b_{77}(x_7)x_7.
\end{aligned} \quad (5)$$

Recall that mass conservation yields $x_3 = \text{MAP2K}_{tot} - x_2 - x_4$, and $x_6 = \text{MAPK}_{tot} - x_5 - x_7$, so we denoted $k = \text{MAP2K}_{tot}$ and $h = \text{MAPK}_{tot}$. Due to space limitations, we only provide an outline of the proofs for propositions 2– 4. The complete proofs can be found in the additional file of [7].

Proposition 2: If $\mu = 0$, x_1 robustly converges to the steady state value, i.e. $\bar{x}_1$ such that $b_{11}(\bar{x}_1)\bar{x}_1 = c_{10}$. Then assuming $\bar{x}_1$ fixed, we can consider the second subsystem Σ_{24} associated with x_2 and x_4. Its steady state conditions are given by

$$\phi_1(\bar{x}_1, x_2, x_4) \doteq -b_{21}(x_2)\bar{x}_1 + c_{23}(k - x_2 - x_4) = 0, \quad (6)$$
$$\phi_2(\bar{x}_1, x_2, x_4) \doteq a_{41}(k - x_2 - x_4)x_1 - b_{44}(x_4)x_4 = 0. \quad (7)$$

The piecewise linear function

$$V(x_2, x_4) = \max\{|x_2 - \bar{x}_2|, |x_4 - \bar{x}_4|, |(x_2 - \bar{x}_2) + (x_4 - \bar{x}_4)|\}$$

is a Lyapunov function for this subsystem; its level surfaces are depicted in Fig. 3.

Therefore, we conclude that the equilibrium point $(\bar{x}_2, \bar{x}_4)$ is stable. It is important to notice that for $\bar{x}_1 \to 0$, $(\bar{x}_2, \bar{x}_4) = (k, 0)$ and for $\bar{x}_1 \to \infty$, $(\bar{x}_2, \bar{x}_4) = (0, k)$. Furthermore, since $(\bar{x}_2, \bar{x}_4)$ are derived implicitly from (6) and (7), from the implicit function theorem we have the following expression for the derivatives of $\bar{x}_2$ and $\bar{x}_4$

[1] This condition is necessarily true for μ small.
[2] I.e. the nullclines have no common tangent lines.

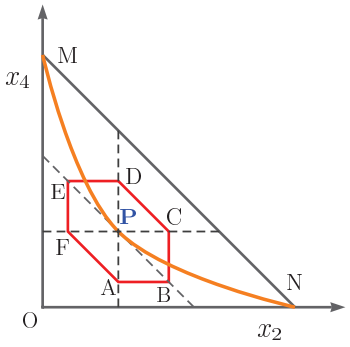

Fig. 3. The x_2–x_4 subsystem

as functions of $\bar{x}_1$

$$\frac{d}{dx_1}\begin{bmatrix} x_2 \\ x_4 \end{bmatrix} = -\begin{bmatrix} -(b'_{21}x_1 + c'_{23}) & -c'_{23} \\ -a'_{41}x_1 & -(a'_{41}x_1 + (b_{44}(x_4)x_4)') \end{bmatrix}^{-1}\begin{bmatrix} -b_{21} \\ a_{41} \end{bmatrix} = \frac{-1}{\Delta_{24}}\begin{bmatrix} -(a'_{41}x_1 + (b_{44}(x_4)x_4)') + c'_{23}) & +c'_{23} \\ +a'_{41}x_1 & -(b'_{21}x_1 + c'_{23}) \end{bmatrix}\begin{bmatrix} -b_{21} \\ a_{41} \end{bmatrix},$$

where Δ_{24} is the determinant of the matrix being inverted. From our assumptions, it can be verified that $\Delta_{24} > 0$. Then the following relation holds for steady state values:

$$\frac{dx_2}{dx_1} = -\frac{(a'_{41}x_1 + (b_{44}(x_4)x_4)')b_{21}(x_2) + c'_{23}a_{41}}{\Delta_{24}} < 0,$$
$$\frac{dx_4}{dx_1} = \frac{a'_{41}x_1b_{21} + (b'_{21}x_1 + c'_{23})a_{41}}{\Delta_{24}} > 0.$$

This implies that the steady state $\bar{x}_2$ decreases when $\bar{x}_1$ increases, while $\bar{x}_4$ increases when $\bar{x}_1$ increases.
The same analysis can be repeated for for the subsystem Σ_{57}, with input x_4. (It is sufficient to replace the indices 2 with 5, and 4 with 7 in all the formulas just derived.) In particular, at steady state we have:

$$\frac{dx_7}{dx_4} = \frac{a'_{74}x_4b_{54} + (b'_{54}x_4 + c'_{56})a_{74}}{\Delta_{57}} > 0.$$

Moreover for a steady state value $\bar{x}_1$, the corresponding value of $\bar{x}_7$ is achieved by a compound function $\varphi : \bar{x}_1 \to \bar{x}_4 \to \bar{x}_7$, namely $\bar{x}_7 = \varphi(\bar{x}_1)$. This function is increasing (both its components are such) and its derivative is

$$\varphi'(x_1) = \frac{dx_7}{dx_4}\frac{dx_4}{dx_1} = \frac{(a'_{41}x_1b_{21} + (b'_{21}x_1 + c'_{23})a_{41})}{\Delta_{24}}\,\frac{(a'_{74}x_4b_{54} + (b'_{54}x_4 + c'_{56})a_{74})}{\Delta_{57}} > 0.$$

Biologically, this means that this cascade model indeed transmits the input signal by increasing the concentration of the active, doubly–phosphorylated species MAP2K–PP and MAPK–PP.

Proposition 3: At steady state $\dot{x}_1 = 0$, $\mu\, a_{17}(x_1)x_7 + c_{10} - b_{11}(x_1)x_1 = 0$. Using the previously defined compound function $x_7 = \varphi(x_1)$, we have the steady state equation:

$$\mu a_{17}(x_1)\varphi(x_1) + c_{10} = b_{11}(x_1)x_1.$$

The terms in the expression above are sketched in Fig. 4. It is apparent that for μ small enough there is an even number of intersections between the two curves. For large values there are no intersections because function $a_{17}(x_1)\varphi(x_1)$ is strictly increasing and $b_{11}(x_1)x_1$ is bounded.

Proposition 4: Consider the linearized system with variables $\delta x_i = x_i - \bar{x}_i$, $i = 1, 2, 4, 5, 7$. The corresponding Jacobian matrix (8) is not a Metzler matrix, but it can be reduced to the Metzler form by the similarity transformation $T^{-1}JT$ with $T = diag\{1, -1, 1, -1, 1\}$. The steady state equation can be written as

$$\psi(x_1) \doteq b_{11}(x_1)x_1 - \mu a_{17}(x_1)\varphi(x_1) - c_{10} = 0,$$

shown in Fig. 4 (blue curve). When $\mu a_{17}(x_1)$ grows unbounded there are only two simple equilibria $\bar{x}_1^A$ and $\bar{x}_1^B$. We have $\psi'(\bar{x}_1^A) > 0$ and $\psi'(\bar{x}_1^B) < 0$. A necessary condition for stability (see Proposition 1) is that the zero degree coefficient of the characteristic polynomial is positive. This condition can be expressed as:

$$\frac{p_0}{\Delta_{24}\Delta_{57}} = (b_{11}x_1)' - \mu a'_{17}\varphi(x_1) - \mu a_{17}\varphi'(x_1) = (b_{11}x_1)' - \frac{d}{dx_1}[\mu a_{17}\varphi(x_1)] = \psi'(x_1),$$

where we recall that Δ_{24} and Δ_{57} are the determinants of the subsystems Σ_{24} and Σ_{57}. Since $\psi'(\bar{x}_1^B) < 0$ we conclude that point B is unstable for $0 < \mu \le \mu^*$.
To show stability of point A we must remember that the opposite condition holds $\psi'(\bar{x}_1^A) > 0$, $0 < \mu \le \mu^*$. Since the eigenvalues depend continuously on the system parameters, we have stability for μ small enough. Moreover, since the Jacobian (8) is similar to a Metzler matrix, in view of Proposition 1, transition to instability (if any) for μ increasing must occur corresponding to $\mu_0 < \mu^*$ in which J has a zero eigenvalue, which implies $p_0 = 0$. Therefore it is impossible that $\psi'(\bar{x}_1^A) = 0$. Hence A must be stable.

We now outline how to prove stability of the equilibria for $a'_{17} \ge 0$. The characteristic polynomial of the system's Jacobian is given by

$$p(s, \mu) = q(s) - \mu\left[a'_{17}x_7r(s) + a_{17}m(s)\right],$$

where $r(s)$ and $m(s)$ are polynomials with positive coefficients, and $q(s) = (s + (b_{11}x_1)')r(s)$.

As already noted, the Jacobian (8) is similar to a Metzler matrix for any $\mu \ge 0$. For $\mu = 0$, $p(s) = q(s)$ has positive coefficients, thus it is stable in view of Proposition 1. Define the following value [3]

$$\mu^* = \inf\{\mu > 0 : p(s, \mu) \text{ has unstable roots}\}.$$

Since the dominant eigenvalue is real, the polynomial at the stability boundary, namely $p(s, \mu^*)$, has a root in zero, say $p(0, \mu^*) = 0$. On the other hand, its constant term is

$$p_0 = q_0 - \mu[a'_{17}x_7r(0) + a_{17}m(0)],$$

which is obviously negative for $\mu > \mu^*$. Therefore the

[3]The parametric study which follows is not affected by the fact that the intersection point is a function of μ and generically valid for any matrix of the form of J

$$J = \begin{bmatrix} -(b_{11}x_1)' + \mu a'_{17}x_7 & 0 & 0 & 0 & \mu a_{17} \\ -b_{21} & -[b'_{21}x_1 + c'_{23}] & -c'_{23} & 0 & 0 \\ a_{41} & -a'_{41}x_1 & -[a'_{41}x_1 + (b_{44}x_4)'] & 0 & 0 \\ 0 & 0 & -b_{54} & -[b'_{54}x_4 + c'_{56}] & -c'_{56} \\ 0 & 0 & a_{74} & -a'_{74}x_4 & -[a'_{74}x_4 + (b_{77}x_7)'] \end{bmatrix} \tag{8}$$

necessary and sufficient condition for stability is

$$p_0 = p(0,\mu) = q_0 - \mu[a'_{17}x_7 r(0) + a_{17}m(0)] > 0.$$

The previously derived expression $p_0/\Delta_{24}\Delta_{57} = \psi'(x_1)$, shows that stability of the equilibrium depends only on the type of intersection. Since in the first intersection point A in Fig. 5 we have $\psi'(x_1^A) > 0$ the first equilibrium is stable and the remaining, alternatively, stable–unstable. The three–point case is depicted in Fig. 5, which represents a bistable situation.

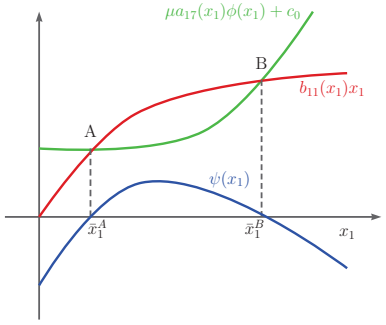

Fig. 4. Functions $\mu a_{17}(x_1)\varphi(x_1) + c_{10}$, $b_{11}(x_1)x_1$ and their difference $\psi(x_1)$.

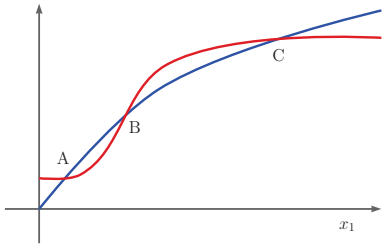

Fig. 5. Functions $\mu a_{17}(x_1)\varphi(x_1)+c_{10}$ (red) and $b_{11}(x_1)x_1$ (blue): the points A and C are stable while B is unstable

V. Conclusions

We have proposed a qualitative model for the MAPK pathway and analyzed its stability properties. Such properties are robustly assured because they do not depend on the specific functional terms or parameters adopted in the model, but rather on the qualitative dynamic relationships between its states. In particular, we have focused on the effects that different positive feedback loop dynamics can have on the equilibria. Our analysis is based on Lyapunov methods, set–invariance theory and matrix theory. Current and future work includes modeling and analysis of other feedback loops that can be present in this pathway and result in different dynamic responses [20].

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