

Relation between intersection of nullclines and periodic solutions in a differential equations of p53 oscillator

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Abstract

We consider a simple mathematical model that suggests emergence of oscillations in p53 and Mdm2 protein levels in response to stress signal. Intracellular activity of the p53 protein is regulated by a transcriptional target, Mdm2, through a feedback loop. The model is classified in five cases with respect to intersection of nullclines. In each case occurrence (or not) of the limit cycle is investigated.

Keywords: DNA damage; p53-Mdm2; Limit cycle; Mathematical biology.

1 Introduction

The p53 protein is suppressor tumor that plays an important role in growth, arrest, senescence, and apoptosis in response to broad array of cellular damage. In more than 50% of human cancer, the p53 is mutated [3]. Under normal, unstressed conditions (for example cells do not suffer DNA damage or no DNA damage) the concentration of p53 is kept at low levels by Mdm2 gene. Mdm2 plays a key role in preserving p53 levels low in normal cell while the Mdm2 transcription is induced by p53 itself [3]. Thus with negative feedback loop ($p53 \rightarrow Mdm2 \dashv p53$) any increase of p53 normally leads to an increase in Mdm2 levels, which then pushes p53 back down to a low steady state level [7]. But in environmental stresses such as DNA damage, the concentration of p53 increases and inducing a transition to oscillations of p53 level [4]. Namely p53 arrests the cell cycle, thereby giving the cell time to correct any DNA damage, activates transcription of genes which is indirectly responsible for DNA repair, and can be the cause of apoptosis [2].

Lahava et al. in [8] measured intracellular concentration of total p53 and Mdm2 protein and observed p53 and Mdm2 protein concentration in a

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single cell oscillation in response to DNA damage, and proposed that system behaved as a digital oscillator [2].

The generation of oscillations in the p53/Mdm2 network seems a challenge to modellers, because negative feedback is not sufficient for oscillatory behaviors. For example, a negative feedback composed of only two elements, such as $p53 \rightarrow Mdm2 \dashv p53$, cannot oscillate. The observation of *Lahava et al.* in [8] leads to several interesting models and hypotheses [3, 9]. In fact several mathematical models have been proposed to explain the damped oscillations of p53, either in cell population or in a single cell, most of which are deterministic models of ordinary differential equations [15]. *Lev Bar Or et al.* considered the possibility of a negative feedback loop composed of three components (Mdm2, p53 and putative intermediate factor), which can oscillate (part A of Figure 1) or require the simultaneous presence of negative and positive feedbacks (part B of Figure 1) [3].

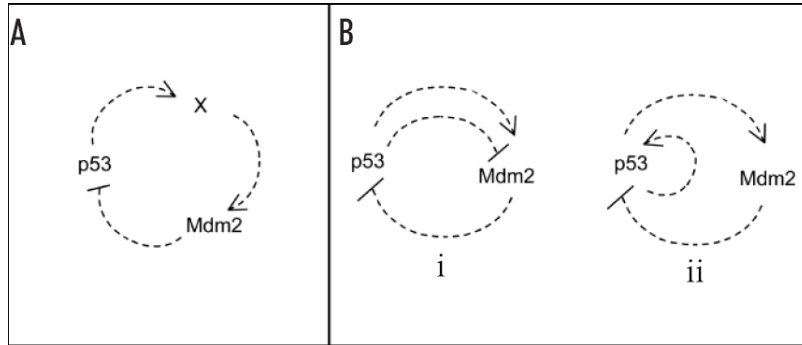


Figure 1: Pathways to oscillations. Oscillation can be found in two different types (A) negative feedback loops with three or more components and (B) combinations of negative and positive feedbacks

Tyson in response to the observation of *Lahava* which showed the digital oscillator behavior, assumed the steady state p53 concentration passes through a Hopf bifurcation in following DNA damage and the p53 and Mdm2 levels begin to oscillate [2, 15]. In [2] another protein (The Atm protein) has been mentioned that is similar to a switch that caused the p53-Mdm2 oscillator to be into or out of oscillatory zone. By following to in [12] regions of parameters into which the Atm protein can switch off damage signals, are determined.

Another approach to modeling the p53 dynamics make explicit use of delays in the system corresponding to the time that it takes for transcription and translation of proteins [10, 14, 16].

Consider two types of motifs, as illustrated in Figure 1, which are discussed in [3]. Case ii in part B of Figure 1 has autocatalysis in p53, whereas in case i of that figure, in addition to the normal activation of Mdm2 by p53, there is a path by which Mdm2 is down-regulated by p53 [2].

A recent elementary model which is motivated biologically according to case ii in part B of Figure1 (autocatalysis) formulates as below

$$\begin{cases} \dot{x} = \alpha_0 + \frac{\alpha_1 x^n}{k_1 + x^n} - \gamma_1 xy - \gamma_2 x = F \\ \dot{y} = \alpha_2 + \frac{\alpha_3 x^4}{k_2 + x^4} - \gamma_3 y = G \end{cases} \quad (1)$$

where $x(t) = [p53(t)]$ and $y(t) = [Mdm2](t)$ are denoting concentration of p53 and Mdm2 respectively[2]. In the first equation above, α_0 shows the production rate of p53, the second term with coefficient α_1 represents an autocatalytic process and it is described with a Hill coefficient $n \in N$ which determines the degree of cooperativity of the ligand of p53 binding to the enzyme or receptor [5]. The third term represents the active process of ubiquitination of p53 independently of Mdm2 and the fourth term represents the degradation of p53 independently of Mdm2. Similarly in the second equation above, α_2 shows the production rate of Mdm2, and second term with coefficient α_3 represents the activation of Mdm2 by p53 with Hill coefficient 4, and the third represents the degradation of Mdm2 [2].

Analysis the trace and determinate of system (1) can be shown in addition to negative feedback loop in p53 - Mdm2 network, autocatalysis by either p53 or Mdm2 leads to the possibility of oscillatory behavior. In the absence of autocatalysis, one can still get oscillations if p53 also down-regulates Mdm2 or Mdm2 also up-regulates p53, in addition to the normal activation.

Here, Oscillatory behavior was described in the form of a limit cycle i.e. to obtain oscillatory behavior from each initial condition the fixed point of the system that resides within the limit cycle needs to be an unstable spiral. In this case all trajectories in the phase plane originating at near that fixed point spiral out and asymptote onto the limit cycle [2].

The goal of system biology is to analyze the behavior and interrelationships of functional biological system [13]. we analyze system (1) to find out the possible cases for existence of limit cycles that oscillatory behavior in p53-Mdm2 network is described. In fact the DNA damage can be controlled when slightly oscillatory region would be given in system (1). In other word, by changing the parameter values (parameter values to get the oscillation) and conditions are imposed on the system (1), then system has the stable limit cycle (oscillatory mode). Therefore, giving cell time to the repair the damage and will not develop cancer. For this purpose we use the Poincare Bendixson Theorem that possible case are shown for existence or nonexistence of limit cycle in system (1). The Poincare Bendixson theorem says that as $t \rightarrow \infty$ the trajectories will tend to a limit cycle solution[11].

Theorem 2. (Poincare Bendixson Theorem) *Suppose that Ω is a nonempty, closed and bounded limit set of a planar of differential equation that contains no equilibrium point. Then Ω is a closed orbit [6].*

On of the most useful tools for analyzing nonlinear systems of differential equations (especially planer systems) are the nullclines. For a system in the form

$$\begin{cases} \dot{x}_1 = f_1(x_1, x_2, \dots, x_n) \\ \vdots \\ \dot{x}_n = f_n(x_1, x_2, \dots, x_n) \end{cases} \quad (2)$$

The x_j -nullcline is the set of points where \dot{x}_j vanishes, so the x_j -nullcline is the set of points determined by setting $f_j(x_1, x_2, \dots, x_n) = 0$ [6].

First, with regard to the intersections of x- and y- nullcline (equilibrium point) of system (1), We classify them in several cases. In any of regions between the nullclines, the vector field is neither vertical nor horizontal, so it must point in one of four direction: northeast, northwest, southeast or southwest. We call such regions **basic region** [6]. The basic regions where $\dot{x} \neq 0$ and $\dot{y} \neq 0$ are of four types:

$$\begin{aligned} & \text{A: } \dot{x} > 0, \dot{y} > 0 \quad \text{B: } \dot{x} < 0, \dot{y} > 0 \\ & \text{C: } \dot{x} < 0, \dot{y} < 0 \quad \text{D: } \dot{x} > 0, \dot{y} < 0 \end{aligned}$$

Equivalently, these are the regions where the vector field points northeast, northwest, southwest, or southeast, respectively. [6].

Next we investigate the possibility of existence or nonexistence of limit cycles in system (1) by using Poincare Bendixson theorem. The Poincare Bendixson theorem says when the trajectory will tend to a limit cycle solution as $t \rightarrow \infty$.

2 Classifying of nullclines

x- nullcline in system (1) is the set of points where $F(x, y) = 0$.

$$F(x, y) = 0 \implies y = f(x) = \frac{1}{\gamma_1} \left(\frac{\alpha_0}{x} + \frac{\alpha_1 x^{n-1}}{k_1 + x^n} - \gamma_2 \right) \quad (3)$$

The map $y = f(x)$ has two critical point

$$x_{1,2} = \sqrt[n]{\frac{k_1(-2\alpha_0 + \alpha_1(n-1) \pm \sqrt{q})}{2(\alpha_0 + \alpha_1)}} \quad (4)$$

where

$$q = -4n\alpha_0 + \alpha_1^2 n^2 - 2\alpha_1^2 n + \alpha_1^2 \quad (5)$$

If we assume that x_1 and x_2 are positive, real and different; Therefore q must be positive. It is easy to see that $x_1 > x_2$.

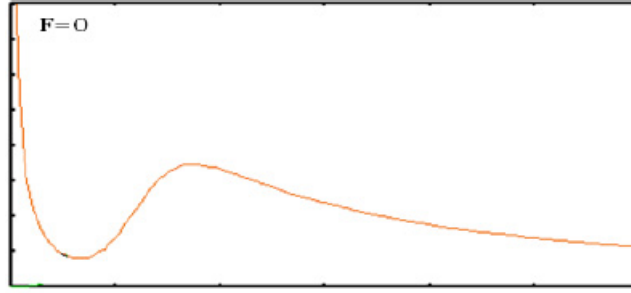


Figure 2: x-nullcline

So if $x \rightarrow 0$, then $f(x) = y \rightarrow \infty$. Since the x_1 and x_2 are positive and $x_1 > x_2$ then sign f' is negative in interval $(0, x_2)$ and the map $y=f(x)$ is decreases in this area. The sign of f' is changed in x_2 because x_1 and x_2 are simple roots of equation $y = f'(x)$. Therefore sign f' is positive between x_1 and x_2 and the map $y=f(x)$ is increases in (x_2, x_1) . The map has a change of sign in x_1 , so sing f' is negative in (x_1, ∞) and $y = f(x)$ is decreasing in this interval. Therefore x_1 and x_2 are maximum and minimum for $y = f(x)$, respectively.

Also, for the graph of $G(x,y)=0$ i.e. y - nullcline We have

$$G(x, y) = 0 \implies y = g(x) = \frac{1}{\gamma_3} \left(\frac{\alpha_3 x^4}{k_2 + x^4} + \alpha_2 \right) \quad (6)$$

and

$$\lim_{x \rightarrow 0} g(x) = \frac{\alpha_2}{\gamma_3}, \quad \lim_{x \rightarrow +\infty} g(x) = \frac{\alpha_3}{\gamma_3} \quad (7)$$

and

$$g'(x) = \frac{1}{\gamma_3} \left(\frac{4\alpha_2 k_2 x^3}{(k_4 + x^4)^2} \right) \quad (8)$$

So $g'(x) > 0$ in the first region coordinate system ($x > 0, y > 0$). Therefore $g(x)$ increases in this region

Now with regard to the intersections of x- and y- nullclines of system (1), it is classified in several cases and we obtained the vector field for each of these cases by XPP software. We discussed possibility of existence or nonexistence of limit cycle near the equilibrium point (intersection of nullclines).

Case 1: The intersection before minimization

In this case, Trajectory of solution tends to intersection point of nullclines and system do not have limit cycle near the intersection point(Figure 4). The vector field is plotted for these parameters in this figure. By the basic region, as mentioned in introduction, the sign of \dot{x} and \dot{y} (F and G) can be determined respectively. We use the direction of vector field and basic regions

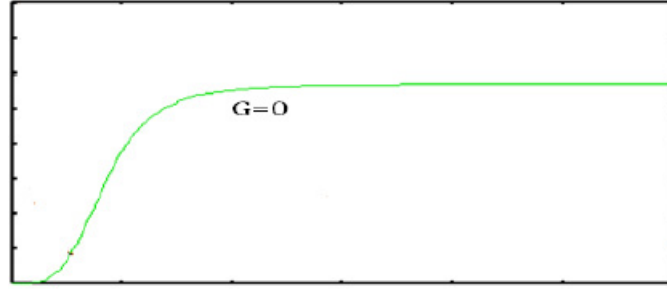


Figure 3: y-nullcline

to get the sing of F_y and F_x and G_x and G_y for Jacobin matrix system(1) in intersection point .

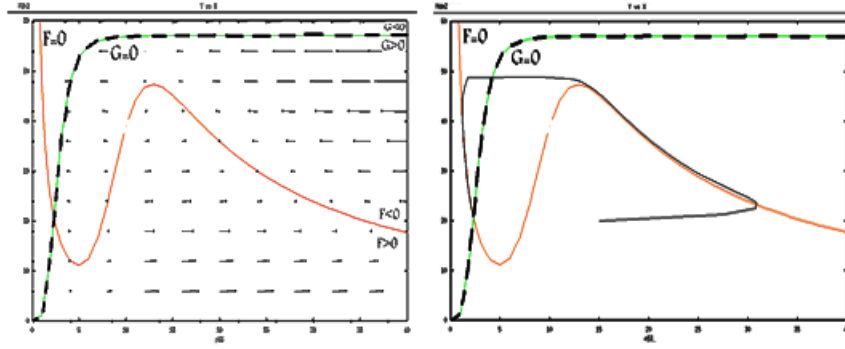


Figure 4: The vector field and nullclines and trajectory solution for the first case

$$\mathbf{A} = \begin{pmatrix} F_x(x_s, y_s) & F_y(x_s, y_s) \\ G_x(x_s, y_s) & G_y(x_s, y_s) \end{pmatrix} \quad (9)$$

We move a long a line parallel to the x -axis through the equilibrium point , F decreases since $F > 0$ the lower x -side and $F < 0$ on the higher x -side. Therefore, Sign of F_x is negative. Similarly, we move a long a line parallel to the y -axis through the equilibrium point , G decreases since $G > 0$ the lower y -side and $G < 0$ on the higher y -side (for detail see [11]). Therefore, Sign of G_y is negative. So x - and y -nullcline is decrease and increase in intersection point respectively. As we have in near the equilibrium point

$$\left. \frac{dx}{dy} \right|_{F=0} = -\frac{F_y}{F_x} < 0, F_x < 0 \Rightarrow F_y < 0 \tag{10}$$

$$\left. \frac{dx}{dy} \right|_{G=0} = -\frac{G_y}{G_x} > 0, G_y < 0 \Rightarrow G_x > 0 \tag{11}$$

In this case, sign of Jacobin matrix is equal

$$\mathbf{A} = \begin{pmatrix} - & - \\ + & - \end{pmatrix} \tag{12}$$

Therefore trace of A matrix is negative and eigenvalues of A matrix is equal

$$\lambda_{1,2} = \frac{trA}{2} \pm \frac{1}{2}\sqrt{(trA)^2 - 4detA} \tag{13}$$

The equilibrium point is stable since eigenvalue of system (1) is negative real part. Therefore system (1) by the Poincare Bendixson Theorem do not have limit cycle near the intersection point in this case.

Case 2: the intersection after maximization of graph F=0

The vector field is plotted in Figure 5. By a similar process in the first case,

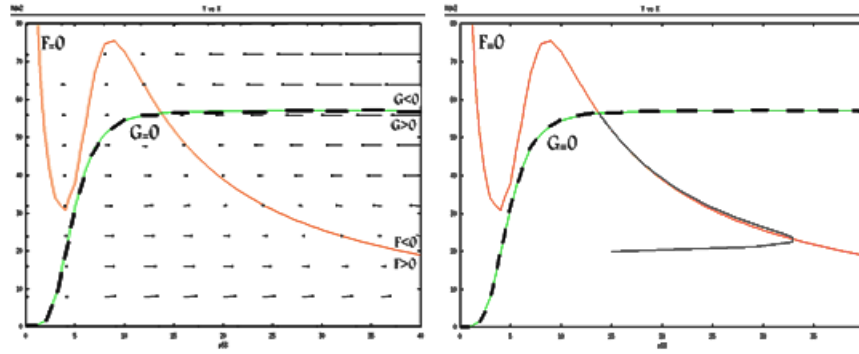


Figure 5: The vector field and nullclines and trajectory solution for the second case

the sign at A matrix is equal

$$\mathbf{A} = \begin{pmatrix} - & - \\ + & - \end{pmatrix} \tag{14}$$

which is $tr\mathbf{A} < 0$ and eigenvalue of system is negative real part. Therefore the equilibrium point is stable. So system (1) do not have limit cycle near the intersection point in this case.

Case 3: the intersection between maximum and minimum of graph $F=0$

Figure 6 shows the vector field in this case. Similarly we have

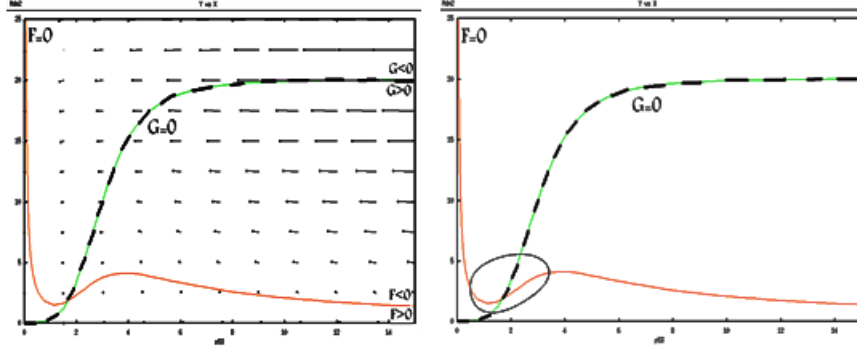


Figure 6: The vector field and nullclines and trajectory solution for the third case

$$\mathbf{A} = \begin{pmatrix} + & - \\ + & - \end{pmatrix} \quad (15)$$

In this case, it is possible that system (1) admits periodic solution [11]. We plotted the limit cycle near the intersection point in Figure 6 .

Case 4: two intersection points

We observe that system has two stable intersection points, which S_1 point is similar to first case. So system (1) has not limit cycle near the intersection point in this case.

Case 5: three intersection points (after maximum, between maximum and minimum, before minimum)

In Figure 8, S_1 and S_3 points are located before of x_2 and after of x_1 respectively. Thus system (1) don't have limit cycle near these points, because S_1 and S_2 are stable points.

For S_2 we have

$$F_x > 0, F_y < 0, G_x > 0, G_y < 0 \quad (16)$$

$$0 < \left. \frac{dy}{dx} \right|_{G=0} < \left. \frac{dy}{dx} \right|_{F=0} \Rightarrow 0 < -\frac{G_x}{G_y} < -\frac{F_x}{F_y}. \quad (17)$$

So

$$\det(A) = F_x G_y - F_y G_x < 0. \quad (18)$$

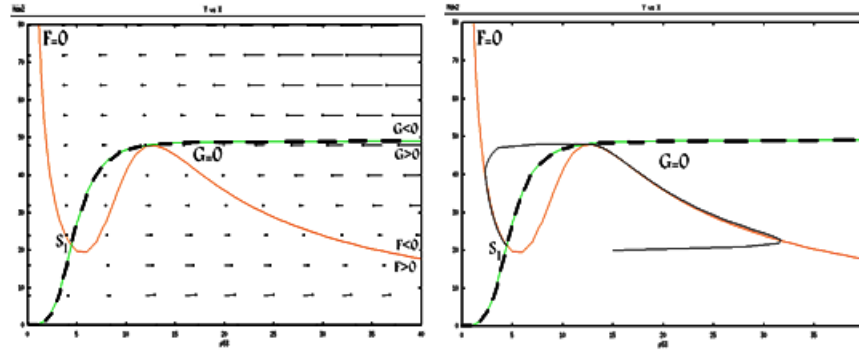


Figure 7: The vector field and nullclines and trajectory solution for the fourth case

This shows that S_2 is saddle point. According to the Poincare Bendixson theorem, this type of singularity does not admit periodic solutions. For more details see([11]Section 7.3)

In following theorem we prove analytically that the only possible case for the existence of limit cycle is second case and other cases don't have limit cycle at all.

Theorem 3. Theorem:System (1) can not admit limit cycle in cases 1, 2, 4 and 5 for the positive value of γ_1, γ_3 and x_s (intersection point of nullclines).

Proof. If (x_s, y_s) is equilibrium point of system (1) then Jacobian matrix of system (1) is equal

$$\mathbf{A} = \begin{pmatrix} F_x(x_s, y_s) & F_y(x_s, y_s) \\ G_x(x_s, y_s) & G_y(x_s, y_s) \end{pmatrix} \quad (19)$$

and eigenvalues of matrix A are

$$\lambda_{1,2} = \frac{trA}{2} \pm \frac{1}{2} \sqrt{(trA)^2 - 4detA} \quad (20)$$

By $G_x = -G_y \frac{dg}{dx}$ and $F_x = -F_y \frac{df}{dx}$ we have

$$det(A) = F_x G_y - F_y G_x = -F_x G_y \frac{df}{dx} + F_y G_y \frac{dg}{dx} \Rightarrow det(A) = F_y G_y (g' - f') \quad (21)$$

Also

$$F_y = -\gamma_1 x, G_y = -\gamma_3 \quad (22)$$

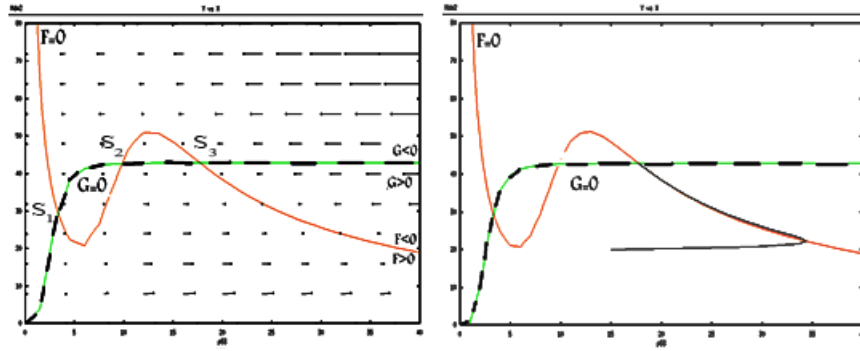


Figure 8: The vector field and nullclines and trajectory solution for the fifth case

Then

$$\lambda_{1,2} = \frac{\gamma_1 x_s f'(x_s) - \gamma_3 \pm \sqrt{(\gamma_1 x_s f'(x_s) - \gamma_3)^2 - 4\gamma_1 \gamma_3 x_s (g'(x_s) - f'(x_s))}}{2} \quad (23)$$

Now since the (x_1, y_1) point is maximum of map f , if the intersection occurs after maximum point then $f'(x_i) \leq 0$ for each of x_i that $x_i \geq x_1$. Hence we have $f'(x_s) \leq 0$, so by the positive value of γ_1, γ_3 and x_s , $trA < 0$ and the (x_s, y_s) point is stable point. Therefore trajectory of solution limits to that point and we don't have limit cycle near this point.

Similarly if the intersection of maps f and g occurs before minimum of map f then the trace of matrix A is negative because of we have $f' \leq 0$ in interval $(0, x_2]$ and so this point is a stable point that trajectory of system (1) limits to that point. \square

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