DYNAMICS AND CODING OF A BIOLOGICALLY-MOTIVATED NETWORK

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A four-node network consisting of a negative circuit controlling a positive one is studied. It models some of the features of the p53 gene network. Using piecewise linear dynamics with thresholds, the allowed dynamical classes are fully characterized and coded. The biologically relevant situations are identified and conclusions drawn concerning the effectiveness of the p53 network as a tumor inhibitor mechanism.

Keywords: Biological networks; p53.

1. Introduction

In the modeling of biological processes, dynamical networks play an important role. Metabolic processes of living beings are networks, the nodes being the substrates, which are linked together whenever they participate in the same biochemical reaction. Protein–protein as well as gene expression and regulation are also dynamical networks.

A central role in the analysis of biological networks is played by the oriented circuits also called *feedback circuits* or *feedback loops* [Snoussi & Thomas, 1993; Thomas *et al.*, 1995]. Feedback circuits are positive or negative according to whether they have an even or odd number of negative interactions. The general understanding is that positive circuits generate multistability and negative circuits generate homeostasis, that is, the variables in the loop tend to middle range values, with or without oscillations [Thomas, 1981; Snoussi, 1998; Gouzé, 1998; Cinquin & Demongeot, 2002; Soulé, 2003].

An interesting situation arises when positive and negative circuits interact and control each other [Kaufman *et al.*, 1985; Kaufman & Thomas, 2001a, 2001b]. Here, one such network is studied. Besides its interest as a dynamical system, it also tries to capture some of the features of the p53 network.

The p53 gene was one of the first tumor-suppressor genes to be identified, its protein acting as an inhibitor of uncontrolled cell growth. The p53 protein has been found not to be acting properly in most human cancers, due either to mutations in the gene or inactivation by viral proteins or inhibiting interactions with other cell products. (There are however a number of cases where

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apparently normal p53 does not achieve tumor control.) Apparently not required for normal growth and development, p53 is critical in the prevention of tumor development, contributing to DNA repair, inhibiting angiogenesis and growth of abnormal or stressed cells [May & May, 1999; Vogelstein *et al.*, 2000; Woods & Vousden, 2001; Taylor *et al.*, 1999; Vousden, 2000]. In addition to its beneficial anticancer activities it may also have some detrimental effects in human aging [Sharpless & DePinho, 2002].

The p53 gene does not act by itself, but through a very complex network of interactions [Kohn, 1999]. The simplified network that is discussed in this paper, although not being accurate in biological detail, tends to capture the essential features of the p53 network as it is known today. In particular, several different products and biological mechanisms are lumped together into a single node when their function is identical. The network is depicted in Fig. 1. The arrows and signs denote the excitatory or inhibitory action of each node on the others and the letters b, c, p, m, g denote their intensities (or concentrations).

The p53 protein (p) is assumed to be produced at a fixed rate and to be degraded after ubiquitin labeling. The MDM2 protein being one of the main enzymes involved in ubiquitin labeling,[1] the inhibitory node is denoted (m). There is a positive feedback link from p53 to MDM2, because the p53 protein, binding to the regulatory region of the MDM2 gene, stimulates the transcription of this gene into mRNA.

Under normal circumstances the network is "off" or operates at a low level. The main activation
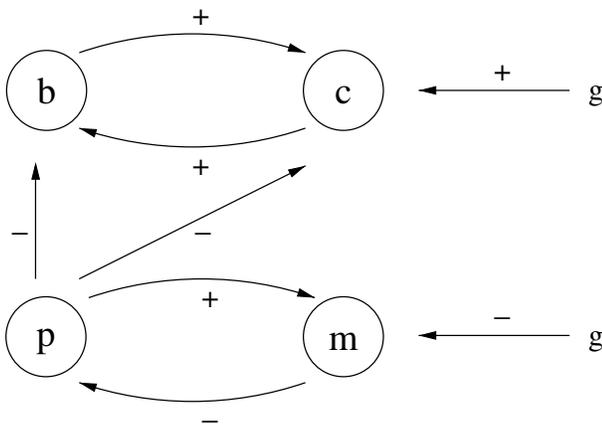

Fig. 1.   A network with a controlled negative circuit controlling a positive one: a simplified p53 network.

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[1]MDM2 binding to p53 can also inhibit p53's transcription.

pathways are the detection of cell anomalies, like DNA damage, or abnormal growth signals, such as those resulting from the expression of several oncogenes. They inhibit the degradation of the p53 protein, which may then reach a high level. There are several distinct activation pathways. For example, in some cases phosphorylation of the p53 protein blocks its interaction with MDM2 and in others it is a protein that binds to MDM2 and inhibits its action. However, the end result being a decrease in the MDM2 efficiency, they may both be described as an inhibitory input control (g) to the MDM2 node.

The p53 protein controls cell growth and proliferation (c), either by blocking the cell division cycle, or activating apoptosis or inhibiting the blood-vessel formation (b).

Blood-vessel formation (b) is stimulated by several tumors and is essential for anomalous cell growth (c). That is, c and b form a positive circuit. Expression of oncogenes or abnormalities is coded as an external control (g) that operates both on the p–m negative circuit and the c–b positive circuit. These two (controlled) circuits will first be studied separately and later their interaction is taken into account.

The dynamical evolution of the network is modeled by a piecewise linear discrete-time system similar to the one used in [Volchenkov & Lima, 2003] for other gene expression networks, namely

$$
\begin{aligned}
p(t+1) &= a_p p(t) + W_{pm} H(T_m - m(t)) \\
m(t+1) &= a_m m(t) + W_{mp} H(p(t) - T_p) \\
&\quad + W_{mg} H(T_g - g) \\
c(t+1) &= a_c c(t) + W_{cb} H(b(t) - T_b) \\
&\quad + W_{cp} H(T_p - p(t)) + W_{cg} H(g - T_g) \\
b(t+1) &= a_b b(t) + W_{bc} H(c(t) - T_c) \\
&\quad + W_{bp} H(T_p - p(t))
\end{aligned}
\tag{1}
$$

H is the Heaviside function, T_i's are the thresholds and in all cases

$$
a_i + \sum_k W_{ik} = 1
\tag{2}
$$

$W_{ik} > 0$. This condition insures that all intensities remain in interval $[0, 1]$.

A similar network was studied in [Vilela Mendes, 2005] modeled by a differential equation system. Both differential and discrete-time systems with thresholds, as opposed to purely logical schemes, automatically include in the dynamics

the action delays characteristic of biological processes. The actual biological process is, of course, a continuous time process. In this sense our model represents a stroboscopic image of the physical process. The main justification to use a piecewise linear discrete-time model is that it allows for a complete mathematical characterization of the dynamical solutions and how they depend on the parameter values.

The study that we perform analyses the two basic network modules, namely the positive and negative circuits. This type of modular analysis has been proposed before [Thieffry & Romero, 1999] as a tool to study biological networks. However this is an adequate methodology only if, in each case, the effect of the other modules is taken into account as an external control. This changes the usual conditions for multistationarity and stable periodicity. We will follow this extended modular approach, by considering the p–m circuit as controlled by the g signal and the c–b circuit as doubly controlled by g and p.

A characterization is obtained of the dynamical regimes of the controlled p–m and c–b circuits and of the coupled system in biologically relevant conditions. The main biological significance of the results might be the identification of the dynamical role of the thresholds as a guide for therapeutic action and a possible dynamical reason for the eventual failure of genetically normal p53 (see Remarks and Conclusions).

2. The p–m System: A Controlled Negative Circuit

$$p(t+1) = a_p p(t) + W_{pm} H(T_m - m(t))$$
$$m(t+1) = a_m m(t) + W_{mp} H(p(t) - T_p) \quad (3)$$
$$+ W_{mg} H(T_g - g)$$

The thresholds T_p and T_m divide the unit square $[0,1]^2$ into four distinct regions with different dynamical laws. This is illustrated in Fig. 3 (for the case $g > T_g$), where close to the corner of each region we have drawn the next-time map
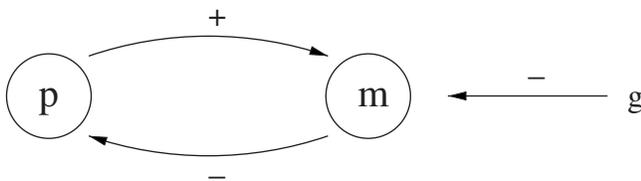

Fig. 2. The p–m system. A controlled negative circuit.

corresponding to the evolution of p and m. Because a_i's are smaller than one, the dynamics tends to drive the system to a fixed point. However if the fixed point of the dynamics is outside that region, the dynamical law will change whenever the system moves to another region. Therefore, the system may never reach a fixed point and evolve forever. This is illustrated in the Fig. 4, where the regions and the corresponding fixed points are color coded for the two cases ($g = 0$ or $g = 1$). Notice also that, for future reference, the four regions are coded as 0, 1, 2 and 3.

Define the quantities

$$fmp = \frac{W_{mp}}{1 - a_m}$$
$$\quad (4)$$
$$fmg = \frac{W_{mg}}{1 - a_m}$$

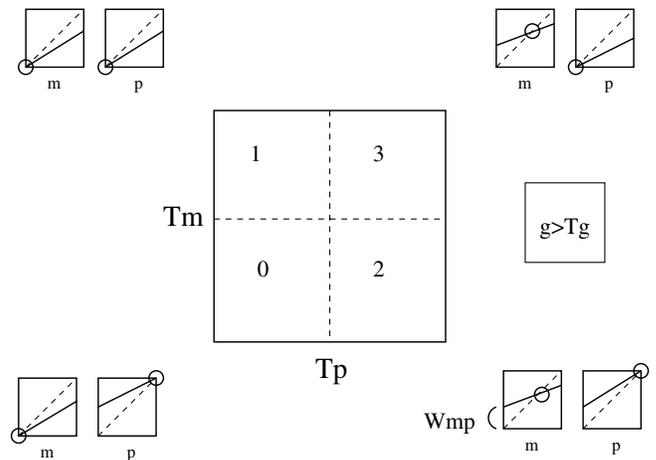
Fig. 3. The p–m system for $g > T_g$. The dynamical laws operating in each region are depicted near their corners.
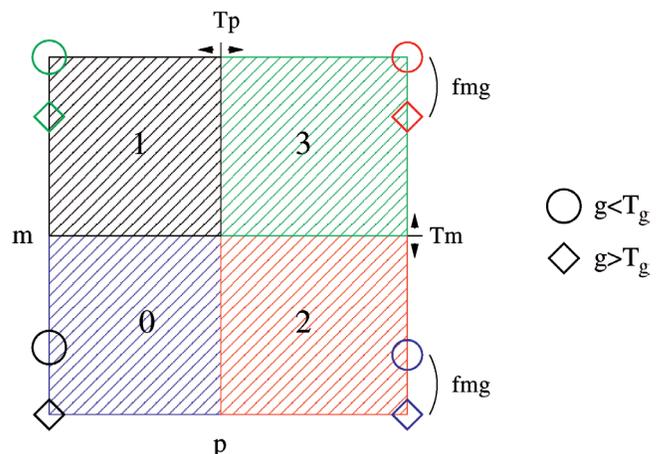

Fig. 4. Color coded dynamical regions and corresponding fixed points for the p–m system.

Notice that from (2) it follows

$$fmp + fmg = 1 \tag{5}$$

Examining Fig. 4 and considering all qualitatively different positions of the thresholds one obtains a complete classification of all possible dynamical behaviors of this system, which are listed in the following table:

Table 1. Asymptotic behavior and coding of the controlled p–m system.

	g	Asympt. behavior	Coding
$T_m < fmg$ $T_m > fmp$	$g < T_g$	$p \to 0, m \to fmg$	$0 \to 3 \to 1$ $\uparrow$ 2
	$g > T_g$	$p \to 1, m \to fmp$	$1 \to 0 \to 2$ $\uparrow$ 3
$T_m < fmg$ $T_m < fmp$	$g < T_g$	$p \to 0, m \to fmg$	$0 \to 3 \to 1$ $\uparrow$ 2
	$g > T_g$	oscillation	$0 \to 2$ $\uparrow \quad \downarrow$ $1 \leftarrow 3$
$T_m > fmg$ $T_m > fmp$	$g < T_g$	oscillation	$0 \to 2$ $\uparrow \quad \downarrow$ $1 \leftarrow 3$
	$g > T_g$	$p \to 1, m \to fmp$	$1 \to 0 \to 2$ $\uparrow$ 3
$T_m > fmg$ $T_m < fmp$	$g < T_g$	oscillation	$0 \to 2$ $\uparrow \quad \downarrow$ $1 \leftarrow 3$
	$g > T_g$	oscillation	$0 \to 2$ $\uparrow \quad \downarrow$ $1 \leftarrow 3$

$$\tag{6}$$

The period of the oscillations will depend on the specific values of the parameters, but the qualitative nature of the dynamics only depends on the relative positions of the thresholds and the quantities fmg and fmp. The coding represents the transitions between the regions defined by the thresholds. Depending on the numerical values of the parameters, the system may stay for a number of time steps in each region but then it is forced to follow the arrows in the coding scheme.

Of the above four working regimens, the first two $(T_m < fmg)$ are the biologically relevant ones, because in the absence of oncogenes (g), normal p53 is expected to be at a low level. The other two regimens correspond to biologically detrimental action of this gene, provoking premature aging.

Oscillations in the p53-MDM2 in response to a stress condition had been considered before [Bar-Or et al., 2000; Brewer, 2002]. Our conclusion is that, in the biological normal region $(T_m < fmg)$, a steady response is also possible if $T_m > fmp$.

3. The c–b System: A Doubly Controlled Positive Circuit

$$
\begin{aligned}
b(t+1) &= a_b b(t) + W_{bc} H(c(t) - T_c) \\
&\quad + W_{bp} H(T_p - p(t)) \\
c(t+1) &= a_c c(t) + W_{cb} H(b(t) - T_b) \\
&\quad + W_{cp} H(T_p - p(t)) + W_{cg} H(g - T_g)
\end{aligned} \tag{7}
$$

Here we consider this system as a positive circuit, with dynamical variables b and c and two controls p and g. The relevant quantities are

$$fcp = \frac{W_{cp}}{1 - a_c}$$

$$fcb = \frac{W_{cb}}{1 - a_c} \quad (8)$$

$$fcg = \frac{W_{cg}}{1 - a_c}$$

with

$$fcp + fcb + fcg = 1 \quad (9)$$

and

$$fbc = \frac{W_{bc}}{1 - a_b}$$

$$fbp = \frac{W_{bp}}{1 - a_b} \quad (10)$$

with

$$fbc + fbp = 1 \quad (11)$$

As before, the dynamics is characterized by analyzing the position of the fixed points for the four possible situations ($p \lessgtr T_p, g \lessgtr T_g$). This is illustrated in Fig. 6, for particular values of the thresholds. The regions defined by the thresholds are, in this case, coded as a, b, c, d. We recall that the coding, listed in the tables, represents the required dynamical transitions between the regions. Depending on the numerical values of the parameters, the system may remain in each region a certain number of time steps before each transition. By adjusting the parameters we may therefore model different action delays in the network. The most important parameters controlling the delays are the dissipation parameters a_i.

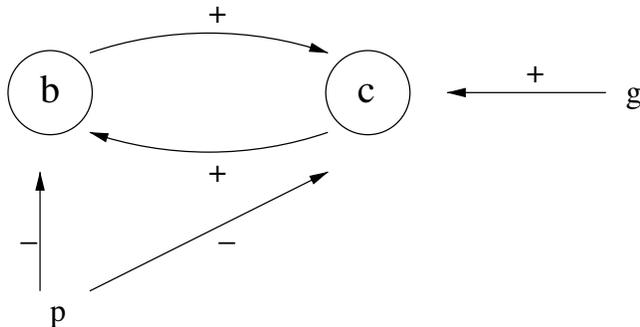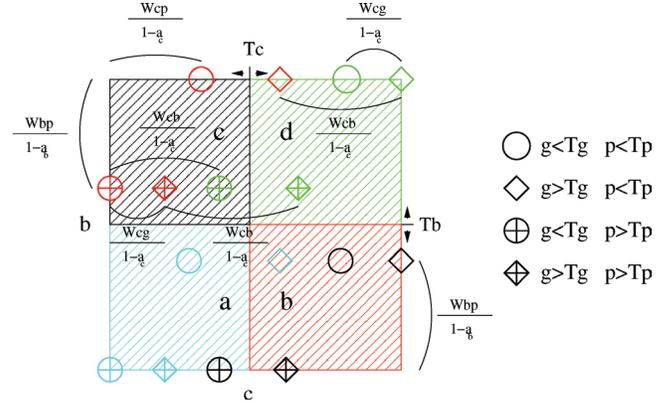Notice that the oscillations coded as b $\leftrightarrows$ c can only be realized if the dynamics jumps directly

Fig. 6. Color coded dynamical regions and corresponding fixed points for the c–b system.

between these two regions. Otherwise, if in the course of the dynamical evolution, the system crosses the regions a or d, it will be attracted to their fixed points. Therefore this oscillation might only be implemented for exceptional parameter values. The emergence of these oscillations results from the simultaneous updating of the variables at discrete times. In the corresponding continuous-time system, of which the discrete-time one is a stroboscopic image, only the two fixed points are attractors because the oscillations are unstable solutions.

4. Biological Implications and the Coupled System

The analysis performed in Secs. 2 and 3 is quite general and applies to any parameter region. However, not all parameter values will model biologically normal conditions. For example, in the absence of cell abnormalities or oncogenes ($g < T_g$), normal p53 will be at a low level. Therefore, as follows from Table 1,

$$T_m < fmg \quad (12)$$

On the other hand, in the absence of oncogenes and low p53, that is, under nonpathological conditions, cell growth should not be explosive. Therefore this condition requires for the model

$$T_c > fcp + fcb$$
$$T_b > fbp \quad (13)$$

as follows from Table 2(a).

With the above (normal condition) requirements we are now ready to analyze the behavior for the other regimens. First, it follows from (13) that

Fig. 5. The c–b system: a doubly controlled positive circuit.

$T_c > fcb$ also. Therefore one sees, from Table 2(b) that if $g < T_g$ and $p > T_p$, then $b \to 0$ and $c \to 0$. That is, in the absence of oncogenes, expression of p53 completely blocks cell growth.

On the other hand, one notices, from Table 2(c), that in all cases, when $g > T_g$ (presence of oncogenes) and $p < T_p$, there is explosive cell growth ($c \to 1$). Only in the special case $T_c > fcg + fcp$ and $T_b > fbp$ there are other solutions, but even in this case $c \to 1$ is a possible solution.

Finally, the most interesting situation is to analyze the effect of expressed p53 ($p > T_p$) in the presence of oncogenes ($g > T_g$) — Table 2(d):

- For $T_c > fcg + fcb$ (and any T_b) p53 effectively controls cell growth, keeping it at a low level ($c \to fcg$). In this network model, a large value of T_c is related to the effectiveness of cell growth to stimulate the required increased level of blood supply. The larger T_c the less effective is the stimulation signal.

Table 2(a).   Case $g < T_g$ and $p < T_p$.

$g < T_g,\ p < T_p$	Asympt. behavior	Coding
$T_c < fcp$ $T_b < fbp$	$b \to 1$ $c \to fcp + fcb$	$a \to d \leftarrow b$ $\uparrow$ c
$T_c < fcp$ $T_b > fbp$	$b \to 1$ $c \to fcp + fcb$	$a \to b \to d$ $\uparrow$ c
$fcp + fcb > T_c > fcp$ $T_b < fbp$	$b \to 1$ $c \to fcp + fcb$	$a \to c \to d$ $\uparrow$ b
$fcp + fcb > T_c > fcp$ $T_b > fbp$	$b \to 1, c \to fcp + fcb$ or $b \to fbp, c \to fcp$ or oscillation	$d \circlearrowleft$ $a \circlearrowleft$ $b \leftrightarrows c$
$T_c > fcp + fcb$ $T_b < fbp$	$b \to fbp$ $c \to fcp + fcb$	$a \to c \leftarrow b$ $\uparrow$ d
$T_c > fcp + fcb$ $T_b > fbp$	$b \to fbp$ $c \to fcp$	$b \to c \to a$ $\uparrow$ d

Table 2(b).   Case $g < T_g$ and $p > T_p$.

$g < T_g,\ p > T_p$	Asympt. behavior	Coding
$T_c < fcb$ $T_b < fbc$	$b \to 0, c \to 0$ or $b \to fbc, c \to fcb$ or oscillation	$a \circlearrowleft$ $d \circlearrowleft$ $b \leftrightarrows c$
$T_c < fcb$ $T_b > fbc$	$b \to 0$ $c \to 0$	$c \to b \to a$ $\uparrow$ d
$T_c > fcb$ $T_b < fbc$	$b \to 0$ $c \to 0$	$b \to c \to a$ $\uparrow$ d
$T_c > fcb$ $T_b > fbc$	$b \to 0$ $c \to 0$	$c \to a \leftarrow b$ $\uparrow$ d

Table 2(c).   Case $g > T_g$ and $p < T_p$.

$g > T_g,\ p < T_p$	Asympt. behavior	Coding
$T_c < fcg + fcp$ $T_b < fbp$	$b \to 1$ $c \to 1$	$c \to d \leftarrow b$ $\uparrow$ a
$T_c < fcg + fcp$ $T_b > fbp$	$b \to 1$ $c \to 1$	$a \to b \to d$ $\uparrow$ c
$T_c > fcg + fcp$ $T_b < fbp$	$b \to 1$ $c \to 1$	$a \to c \to d$ $\uparrow$ b
$T_c > fcg + fcp$ $T_b > fbp$	$b \to 1, c \to 1$ or $b \to fbp, c \to fcg + fcp$ or oscillation	$d \circlearrowleft$ $a \circlearrowleft$ $b \leftrightarrows c$

Table 2(d).   Case $g > T_g$ and $p > T_p$.

$g > T_g,\ p > T_p$	Asympt. behavior	Coding
$T_c < fcg$ $T_b < fbc$	$b \to fbc$ $c \to fcg + fcb$	$a \to b \to d$ $\uparrow$ c
$T_c < fcg$ $T_b > fbc$	$b \to fbc$ $c \to fcg$	$a \to b \leftarrow d$ $\uparrow$ c
$fcg + fcb > T_c > fcg$ $T_b < fbc$	$b \to fbc, c \to fcg + fcb$ or $b \to 0, c \to fcg$ or oscillation	$d \circlearrowleft$ $a \circlearrowleft$ $b \leftrightarrows c$
$fcg + fcb > T_c > fcg$ $T_b > fbc$	$b \to 0$ $c \to fcg$	$c \to b \to a$ $\uparrow$ d
$T_c > fcg + fcb$ $T_b < fbc$	$b \to 0$ $c \to fcg$	$b \to c \to a$ $\uparrow$ d
$T_c > fcg + fcb$ $T_b > fbc$	$b \to 0$ $c \to fcg$	$c \to a \leftarrow b$ $\uparrow$ d

- For T_c values smaller than $fcg + fcb$ the inhibitory effect of p53 may not be so effective. For example, for $fcg + fcb > T_c > fcg$ and $T_b < fbc$ there are several solutions and the outcome will depend on the initial conditions. For $T_c < fcg$ and $T_b < fbc$ no control is possible.

For parameter values of T_m and T_p for which the p–m system is driven towards a fixed point, the behavior of the coupled system may be read directly from the c–b tables of the previous section and the conclusions are as listed above. For oscillatory conditions on the p–m system, the behavior is more complex and several different regimens may be accessed. Here we will illustrate, by direct numerical simulation, the behavior of the coupled system, for regions of parameter values which, as discussed above, are biologically relevant ($T_m < fmg$, $T_c > fcp + fcb$ and $T_b > fbp$).

Figures 7 and 8 show the dynamical evolution of the variables p, m, b and c for $g > T_g$ and the parameter values

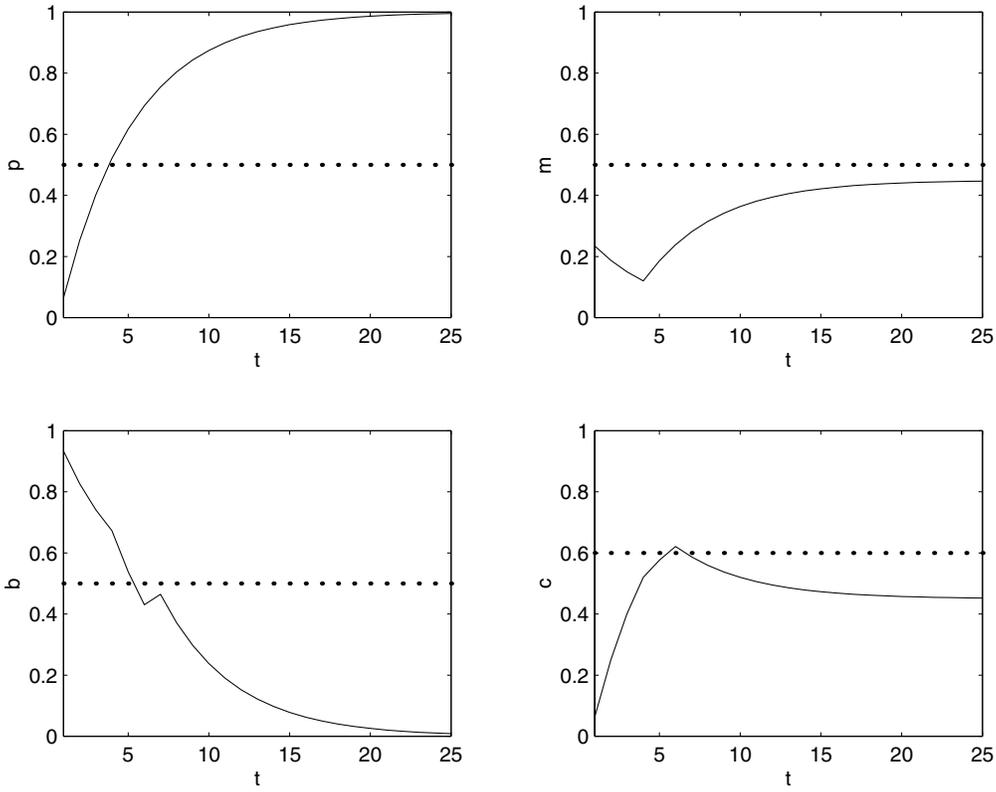$$fmg = 0.55, \quad fbp = 0.4, \quad fcb = 0.35, \quad fcp = 0.2$$

Fig. 7.   Dynamical evolution of p, m, b, and c for $fmg = 0.55$, $fbp = 0.4$, $fcb = 0.35$, $fcp = 0.2$, $a_i = 0.8$, $T_p = 0.5$, $T_m = 0.5$, $T_c = 0.6$, $T_b = 0.5$, $g > T_g$.
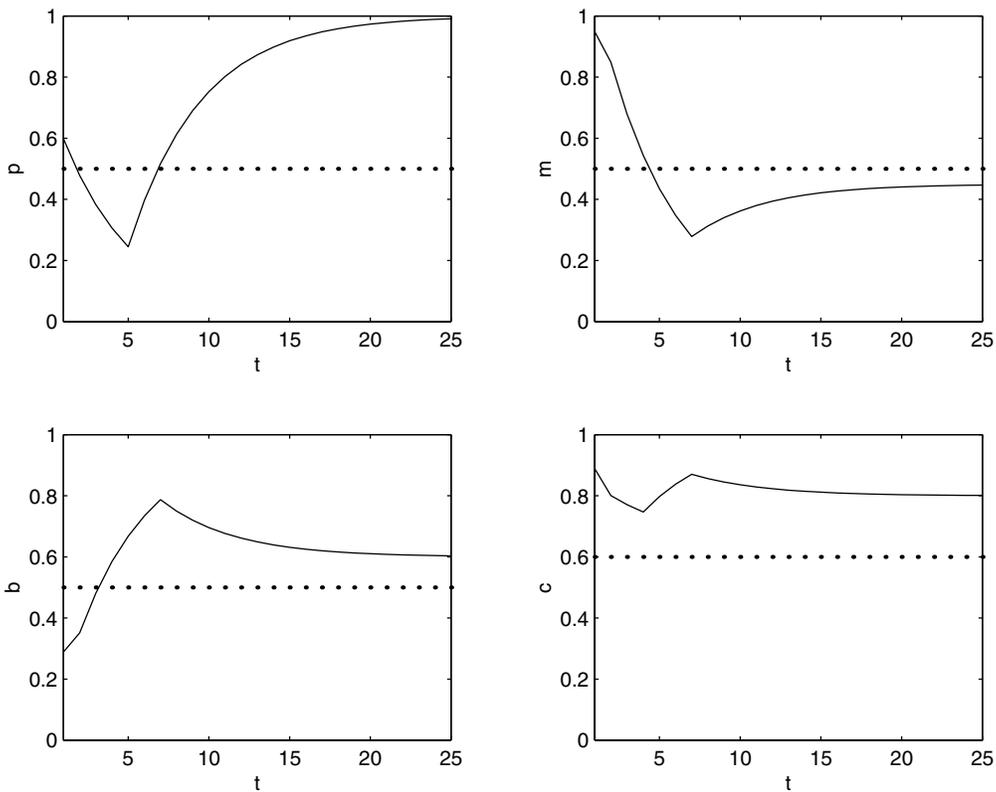

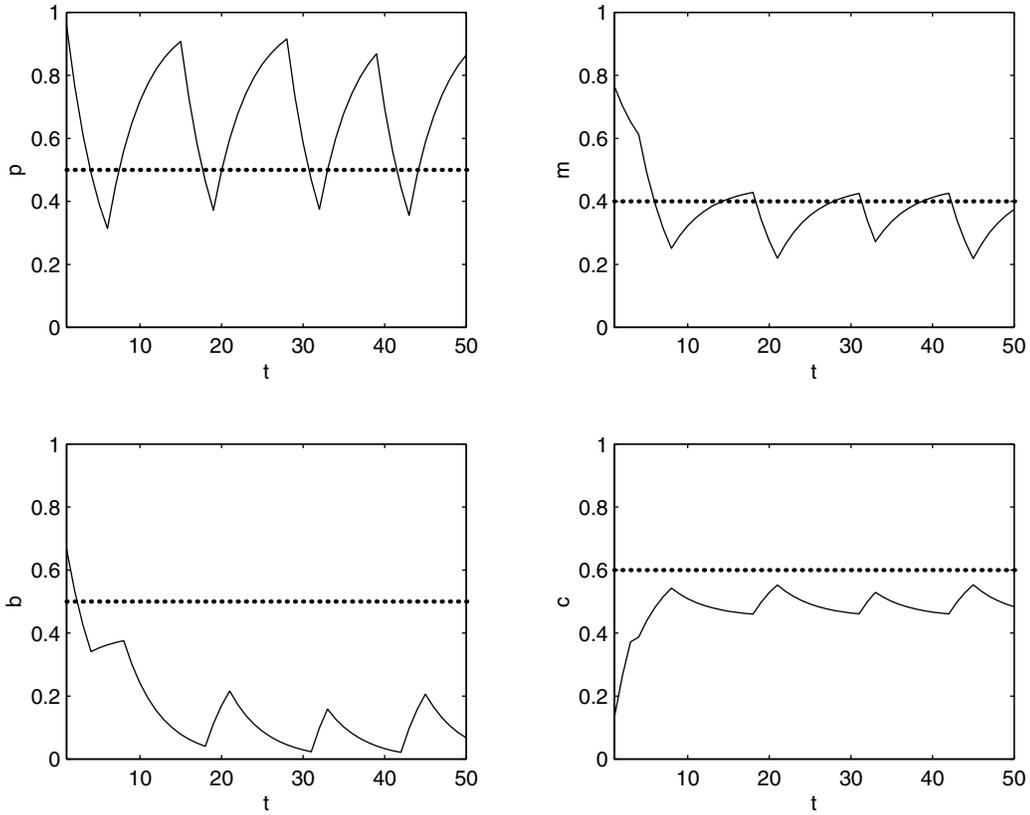Fig. 8.   Same as Fig. 7 with different initial conditions.

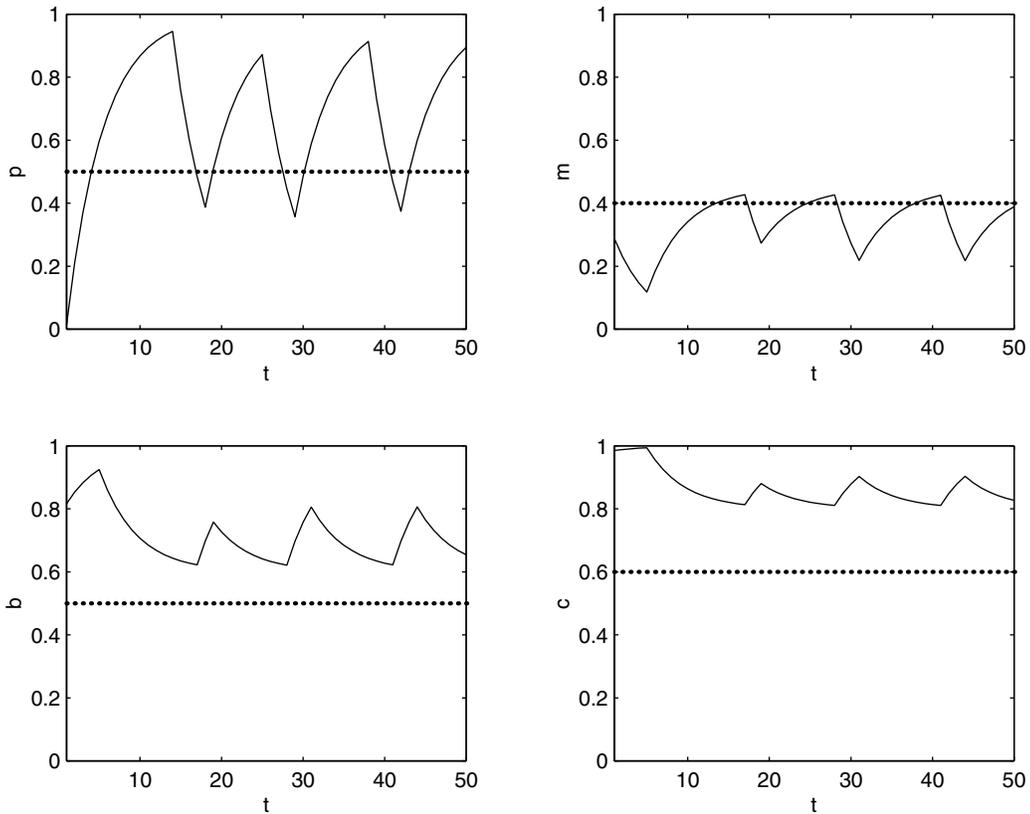Fig. 9.   Same as Fig. 7 with $T_m = 0.4$ instead of $T_m = 0.5$.

Fig. 10.   Same as Fig. 9 with different initial conditions.

and

$$T_p = 0.5, \quad T_m = 0.5, \quad T_c = 0.6, \quad T_b = 0.5$$

This corresponds to the situation

$$T_m < fmg, \quad T_m > fmp$$

in the p–m system and

$$fcg + fcb > T_c > fcg, \quad T_b < fbc$$

in the c–b system.

The parameter values are the same for both figures. One sees that depending on the initial conditions, in one case the p53 action keeps c at a low level and in the other it does not.

Figures 9 and 10 use the same parameters for the c–b system, the only change being the value of T_m that is now 0.4, that is, $T_m < fmg$ and $T_m < fmp$. In this case the p–m system oscillates instead of converging to a fixed point, but the action on the c–b system is similar and it again depends on the initial conditions.

5. Remarks and Conclusions

(i) A simplified four-node model displays biologically reasonable features which might, at least, serve as a toy model for the p53 network. The model allows for a complete mathematical characterization of its dynamical solutions and how they depend on the parameter values (thresholds and couplings). In biology, to change the threshold positions corresponds to stimulate or inhibit the mechanisms of gene expression. Therefore the dynamical identification of their role on the dynamics of the network might be a guide for therapeutic action.

(ii) For very large values of the T_c threshold, our model p53 (p) achieves control of cell growth in the presence of oncogenes. However, as illustrated before, there is a biologically reasonable region of parameters for which the successful action of p, as an inhibitor of cell growth, strongly depends on the initial conditions. In practice it means that it is only effective if the p level is already large enough before the positive feedback effect of c on b starts to play a role.

It is known that in about half of human cancers p53 action is lost through mutation in the p53 gene. In many of the remaining tumors, the p53 gene is intact but it does not achieve the proper response [Woods & Vousden, 2001]. Several mechanisms have been proposed to explain the failure of genetically normal p53. They include abnormal conformation, targeting for degradation by viral proteins, defective localization to the nucleus, amplification of MDM2, loss of ability to inhibit MDM2 through proteins such as p14^{ARF} or loss of kinases that phosphorylate MDM2 and p53. What our simple model suggests is that there is a range of biological parameters for which success or failure of the p53 action is very much a question of chance, that is, it depends on the initial conditions of the process. This would be a purely dynamic explanation for the failure of normal p53.

(iii) The actual biological p53 network is highly complex and contains very many nodes and pathways [Kohn, 1999]. Our toy model has concentrated on extracting the abstract functional actions rather than reproducing the biological detail. However, there are still many other dynamical mechanisms in the organic control of DNA abnormalities or pathological cell proliferation. A number of other genes either play a similar role or interact with p53. On the other hand some of the "defense" mechanisms of cancer cells are directly related to the operation of the basic network. For example, oxygen starving (hypoxia) of tumor cells by lack of blood supply (the b node intensity) activates the CXCR4 gene and this activation causes the tumor cells to migrate to other organs (metastasis) [Staller *et al.*, 2003]. Inclusion of this and other qualitatively different effects seems an interesting possibility.

(iv) Sections 2 and 3 contain a complete classification of the fixed versus periodic nature of the orbits. However, as mentioned before, the actual period of the oscillations depends strongly on the values of the parameters. In Fig. 11 we illustrate this dependence by color coding the logarithm of the period s of the orbits, in the negative circuit, as a function of the parameters a_m and a_p. The parameters are chosen such that $W_{pm} = 1 - a_m$, $W_{mp} = 0.5(1 - a_m)$ and $W_{mg} = 0.5(1 - a_m)$. These conditions satisfy the oscillation condition and maintain normalization.
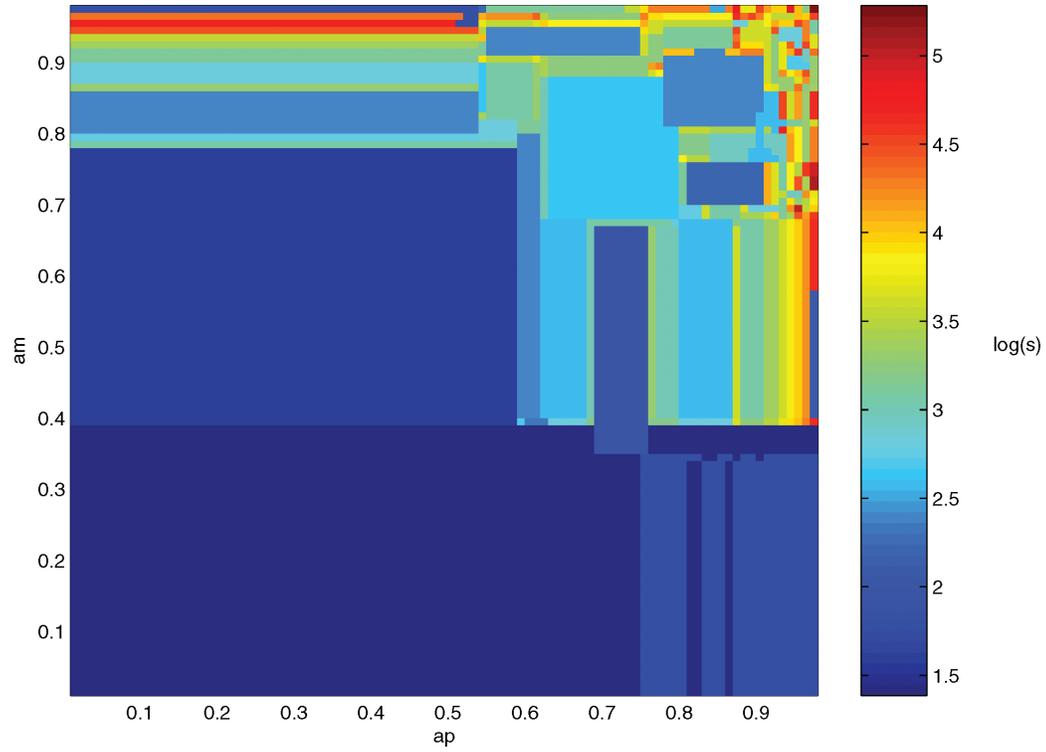
Fig. 11.   Color-coded log-period of the closed orbits in the negative circuit. $g > T_g$, $T_p = 0.5$, $T_m = 0.4$, $fmg = 0.5$.
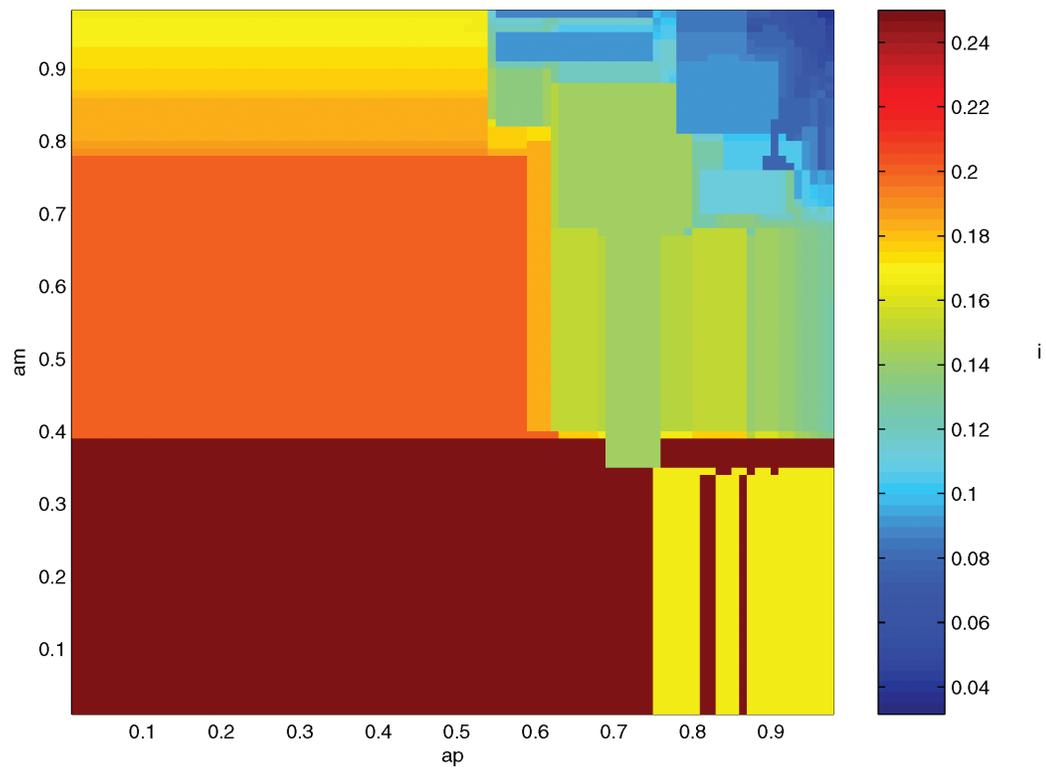

Fig. 12.   Color-coded rotation number of the periodic orbits in the negative circuit. The parameters are the same as in Fig. 11.

To visualize the orbits corresponding to any parameter value a tool for Windows is available for download at http://www.ii.uam.es/aguirre/p53_emu.zip.

The behavior of the periods is rather discontinuous, switching from small to large values for small parameter changes. This occurs, in particular, when some points of the orbit lie close to the thresholds.

In Fig. 12 the rotation number i (the number of cycles divided by the period of the orbit, is depicted. This quantity displays a more continuous behavior that the period of the orbits. Highest values of i correspond to high values of both a_m and a_p. However, there are still some discontinuities due mainly to orbits close to the intersection of the thresholds.

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