Piecewise linear map models for biological networks. The p53 example

Rui Vilela Mendes

Centro de Matemática e Aplicações, Univ. Lisbon
http://label2.ist.utl.pt/vilela/

(work with C. Aguirre and J. Martins)

Introduction

- In modeling of biological processes, dynamical networks play an important role.
  - Metabolic processes, Protein-protein, Gene expression and regulation, etc.
  - A central role: oriented circuits or *feedback loops* [Snoussi & Thomas, 1993], [Thomas et al., 1995]. Positive or negative according to whether they have an even or odd number of negative interactions.
  - General understanding: positive circuits generate multistability and negative circuits generate homeostasis [Snoussi, 1998], [Gouzé, 1998].
  - The difficult cases: positive and negative loops interacting and controling each other

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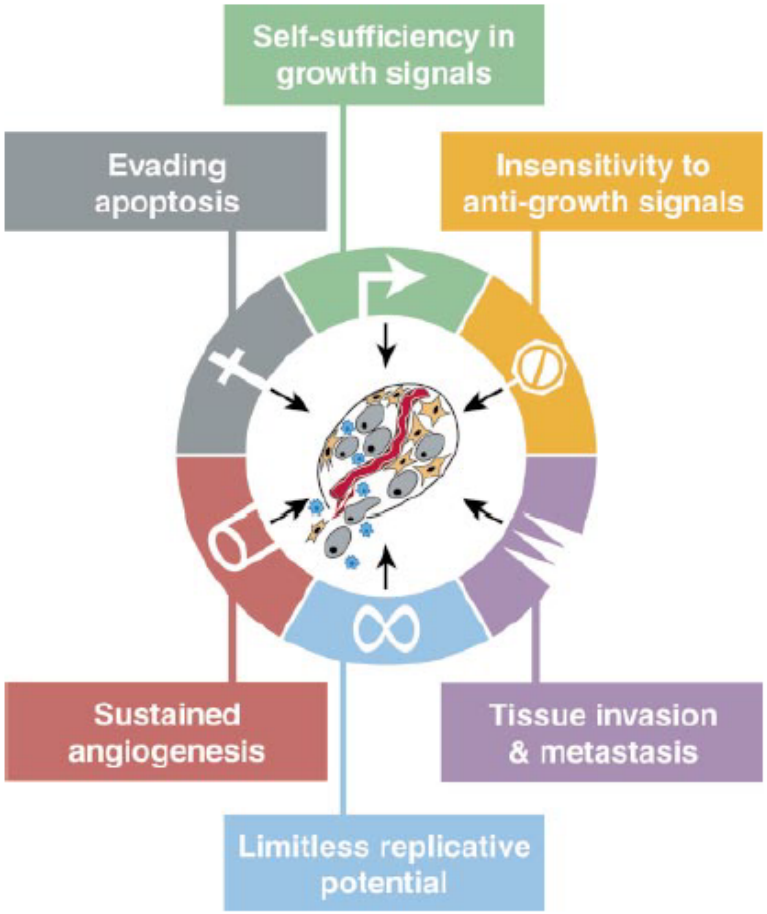
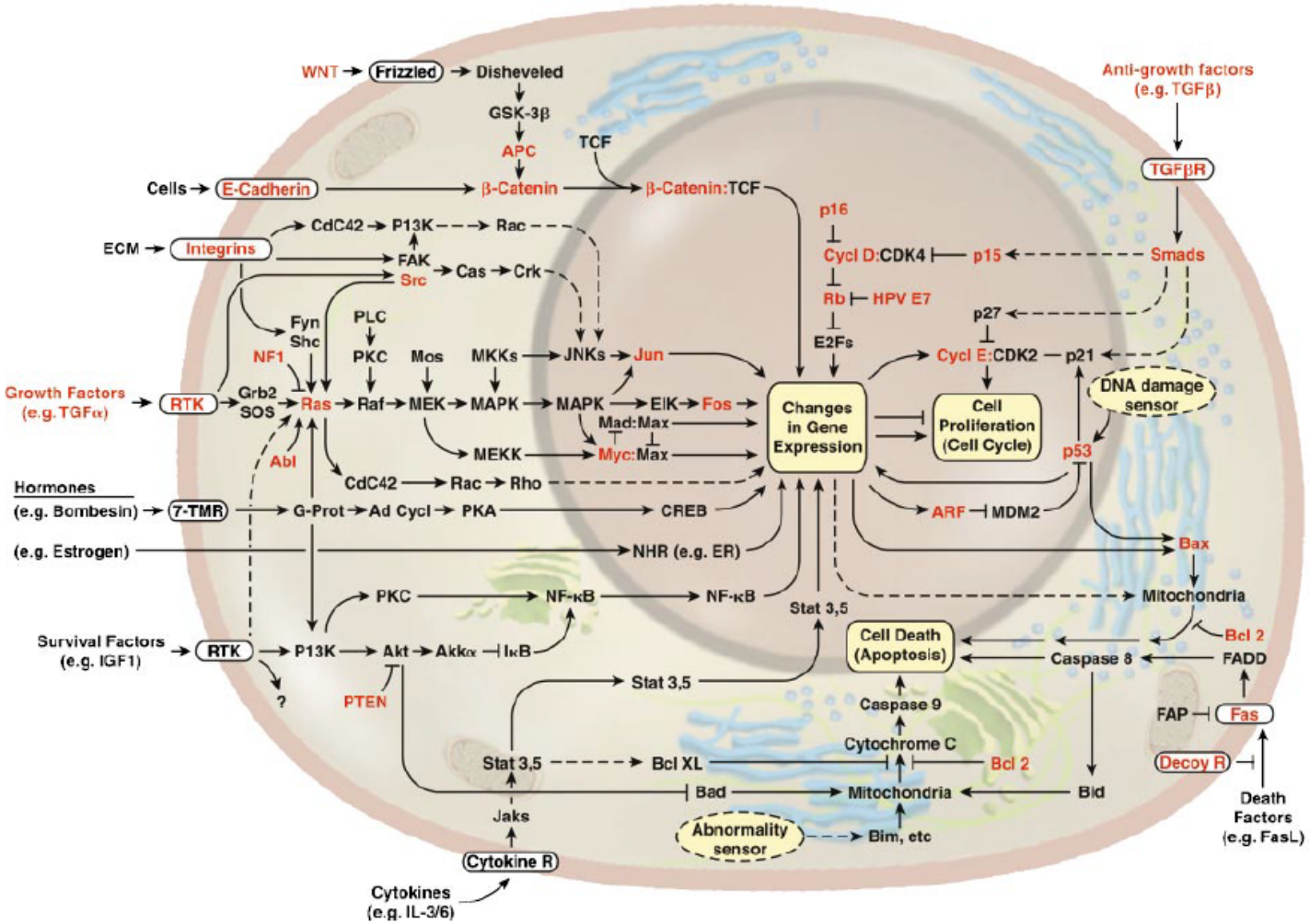
  ■ General understanding: positive circuits generate multistability and negative circuits generate homeostasis [Snoussi, 1998], [Gouzé, 1998].
  ■ The difficult cases: positive and negative loops interacting and controling each other
- **Modeling approaches : (Thresholds and delays)**
  Differential equations (with or without delays)
  The logic approach
  The piecewise linear approach

The acquired capabilities of cancer



The cell development circuitry



The p53 network

- p53 is a tumor suppressor gene. Its protein (transc. activ.) acts by :
  - Inhibition of progress through the cell cycle (p21, CDK's,...)
  - Apoptosis (Bax, NOXA, ...)
  - Inhibition of blood-vessel formation (angiogenic phase)
  - Modulating the balance between respiration and glycolysis

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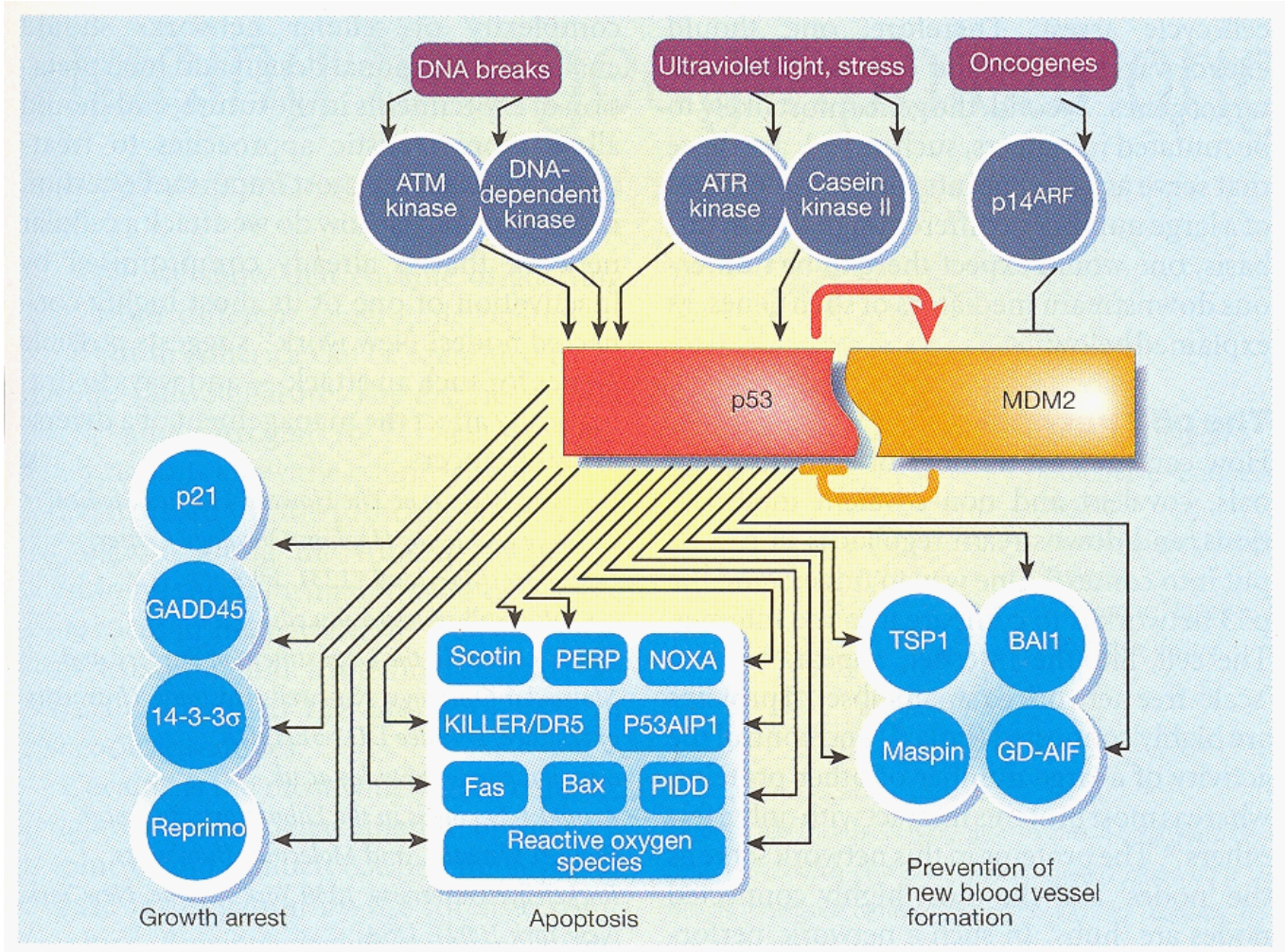
  It activates its own controller
- In many tumors (~50%) it is found to be mutated
  In a number of cases normal p53 cannot achieve control

The p53 network

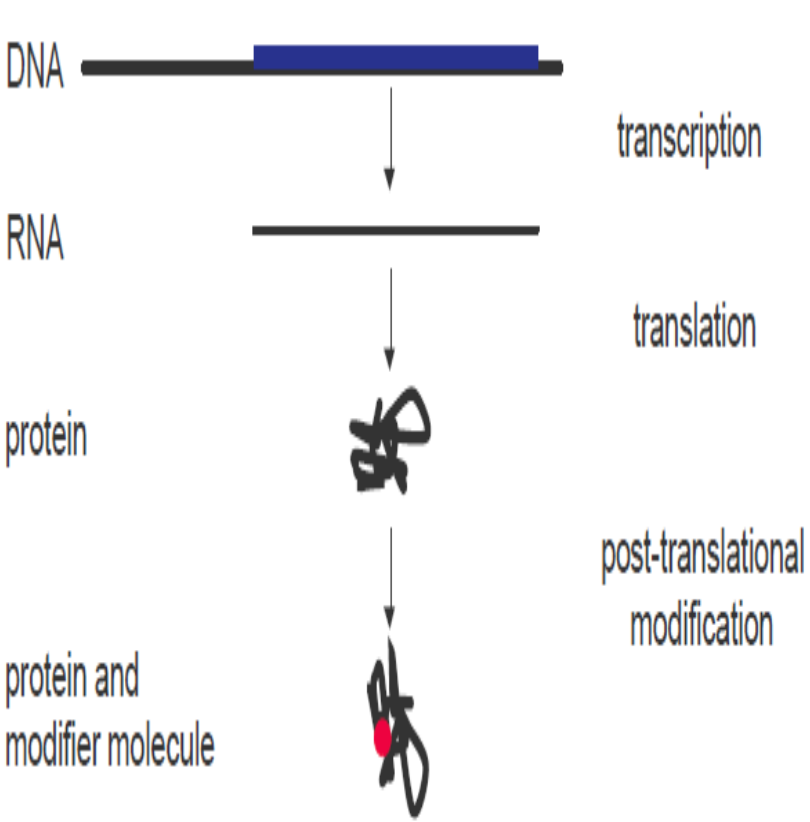


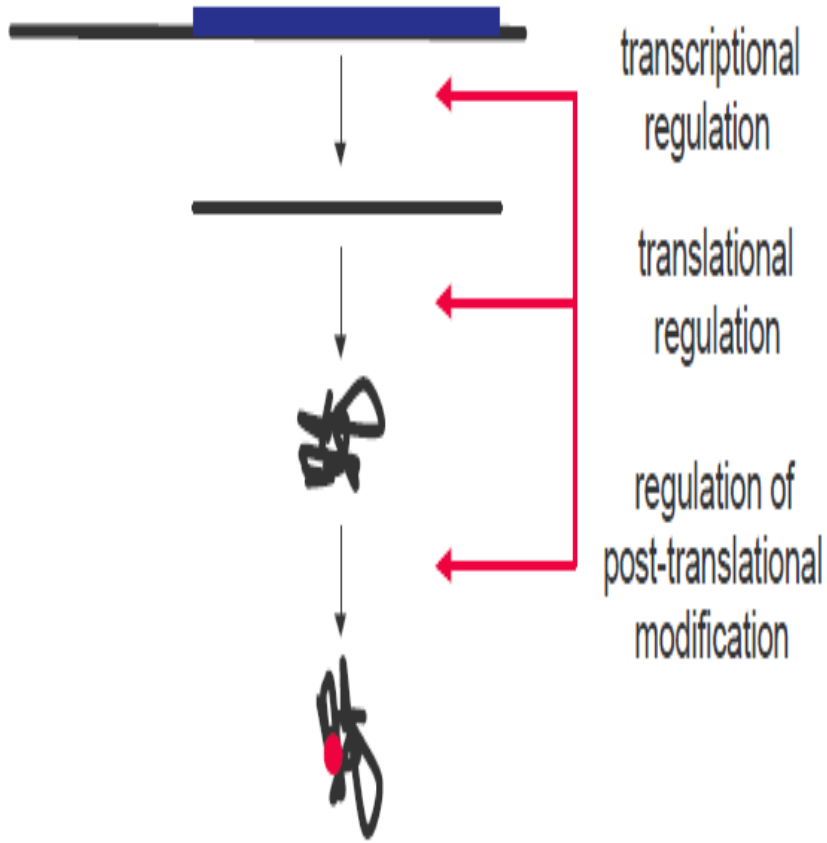
The "normal" inactivation of p53

Mechanism of inactivating p53	Typical tumours	Effect of inactivation
Amino-acid-changing mutation in the DNA-binding domain	Colon, breast, lung, bladder, brain, pancreas, stomach, oesophagus and many others	Prevents p53 from binding to specific DNA sequences and activating the adjacent genes
Deletion of the carboxy-terminal domain	Occasional tumours at many different sites	Prevents the formation of tetramers of p53
Multiplication of the MDM2 gene in the genome	Sarcomas, brain	Extra MDM2 stimulates the degradation of p53
Viral infection	Cervix, liver, lymphomas	Products of viral oncogenes bind to and inactivate p53 in the cell, in some cases stimulating p53 degradation
Deletion of the $p14^{ARF}$ gene	Breast, brain, lung and others, expecially when p53 itself is not mutated	Failure to inhibit MDM2 and keep p53 degradation under control
Mislocalization of p53 to the cytoplasm, outside the nucleus	Breast, neuroblastomas	Lack of p53 function (p53 functions only in the nucleus)

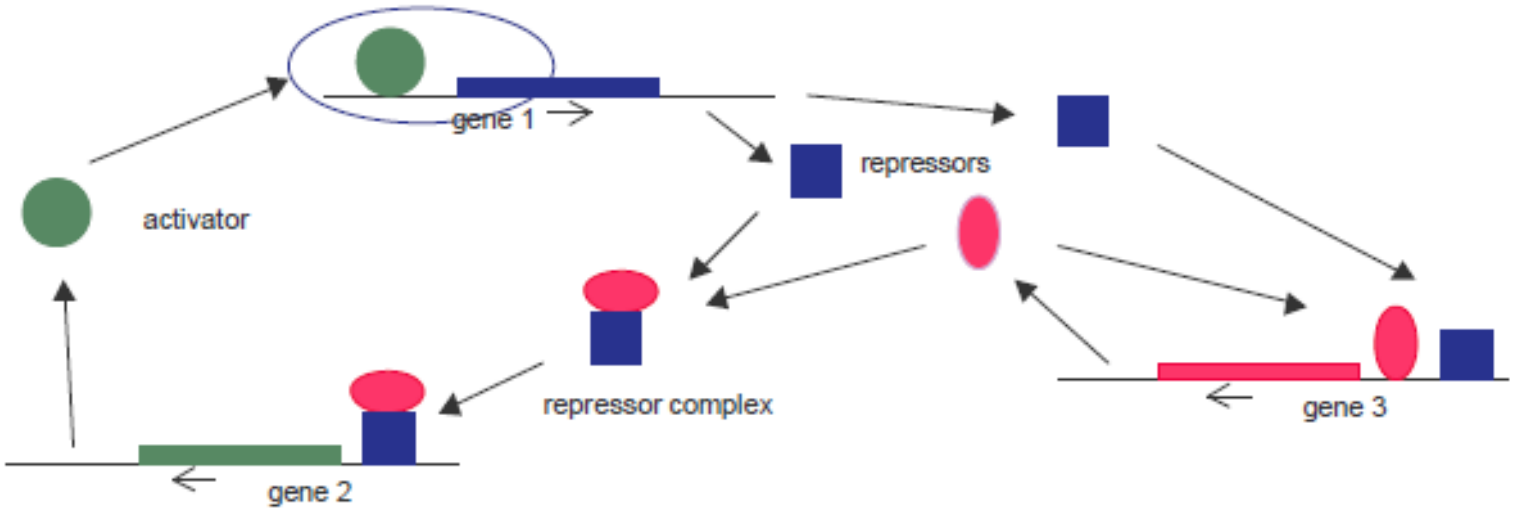
The research motivation: Malfunction of wild type p53. (The other ~50% cases)

The complexity of gene expression regulation

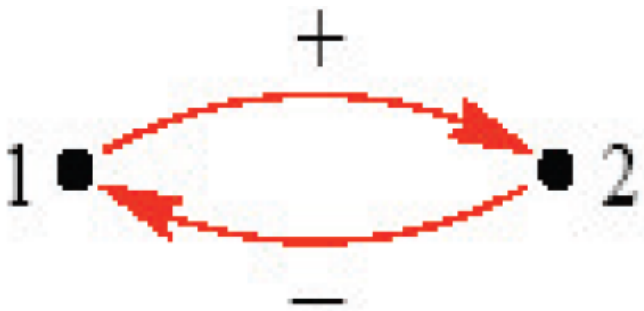


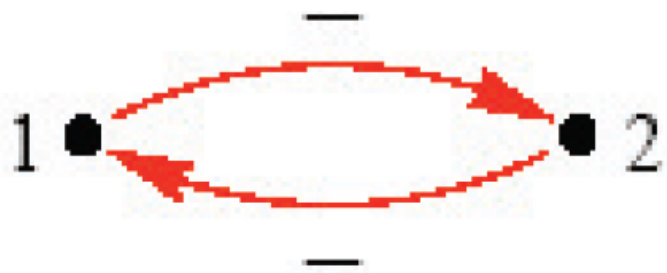


The modular approach: Positive and negative circuits



Negative and positive circuits

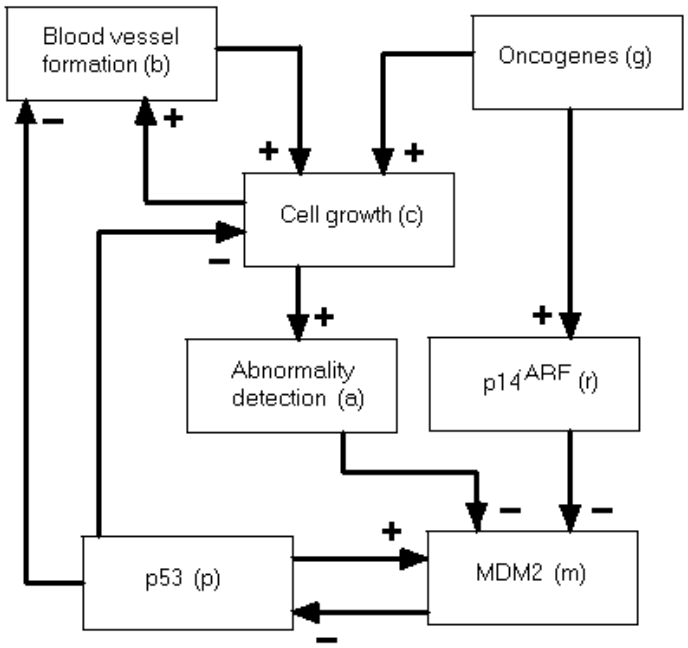




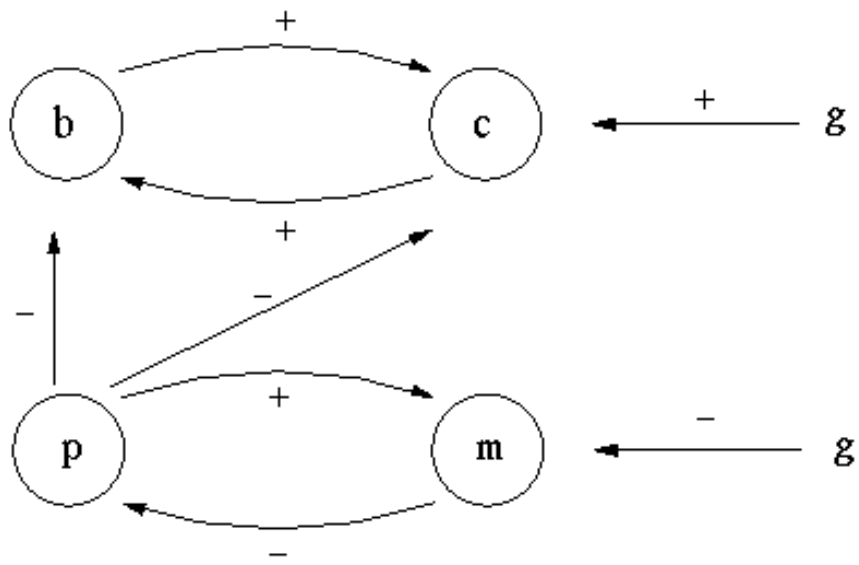
The Thomas rules

- When a gene network has several non-degenerate steady states, the corresponding interaction graph has to contain, somewhere in the graph, a positive circuit.
  Positive circuit is necessary for multistability
- When a gene network has oscillations, the corresponding interaction graph has to contain, somewhere in the graph, a negative circuit.
  Negative circuit is necessary for oscillations

A model for the p53 network



Controlled positive and negative circuits in interaction



The equations: Piecewise linear maps

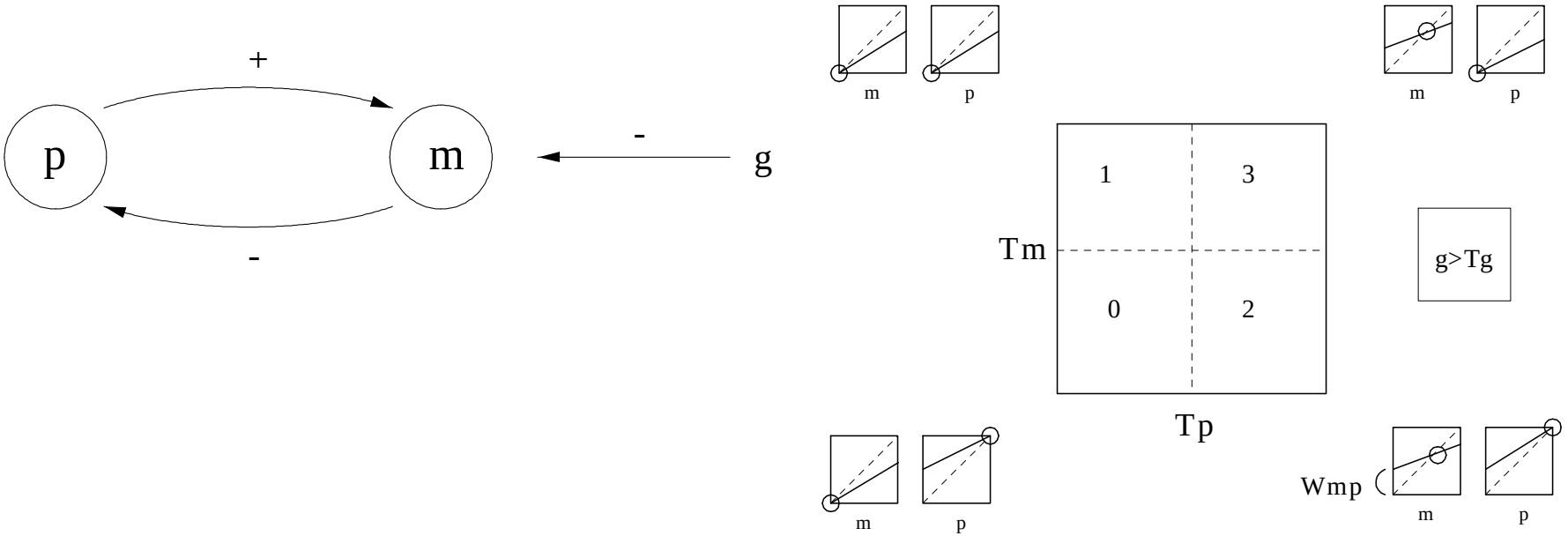
$$
\begin{aligned}
p(t+1) &= a_p p(t) + W_{pm} H(T_m - m(t)) \\
m(t+1) &= a_m m(t) + W_{mp} H(p(t) - T_p) + W_{mg} H(T_g - g) \\
c(t+1) &= a_c c(t) + W_{cb} H(b(t) - T_b) + W_{cp} H(T_p - p(t)) \\
&\quad + W_{cg} H(g - T_g) \\
b(t+1) &= a_b b(t) + W_{bc} H(c(t) - T_c) + W_{bp} H(T_p - p(t))
\end{aligned}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

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

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

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

The p-m system: a controlled negative circuit



$$
\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) + W_{mg} H(T_g - g)
\end{aligned}
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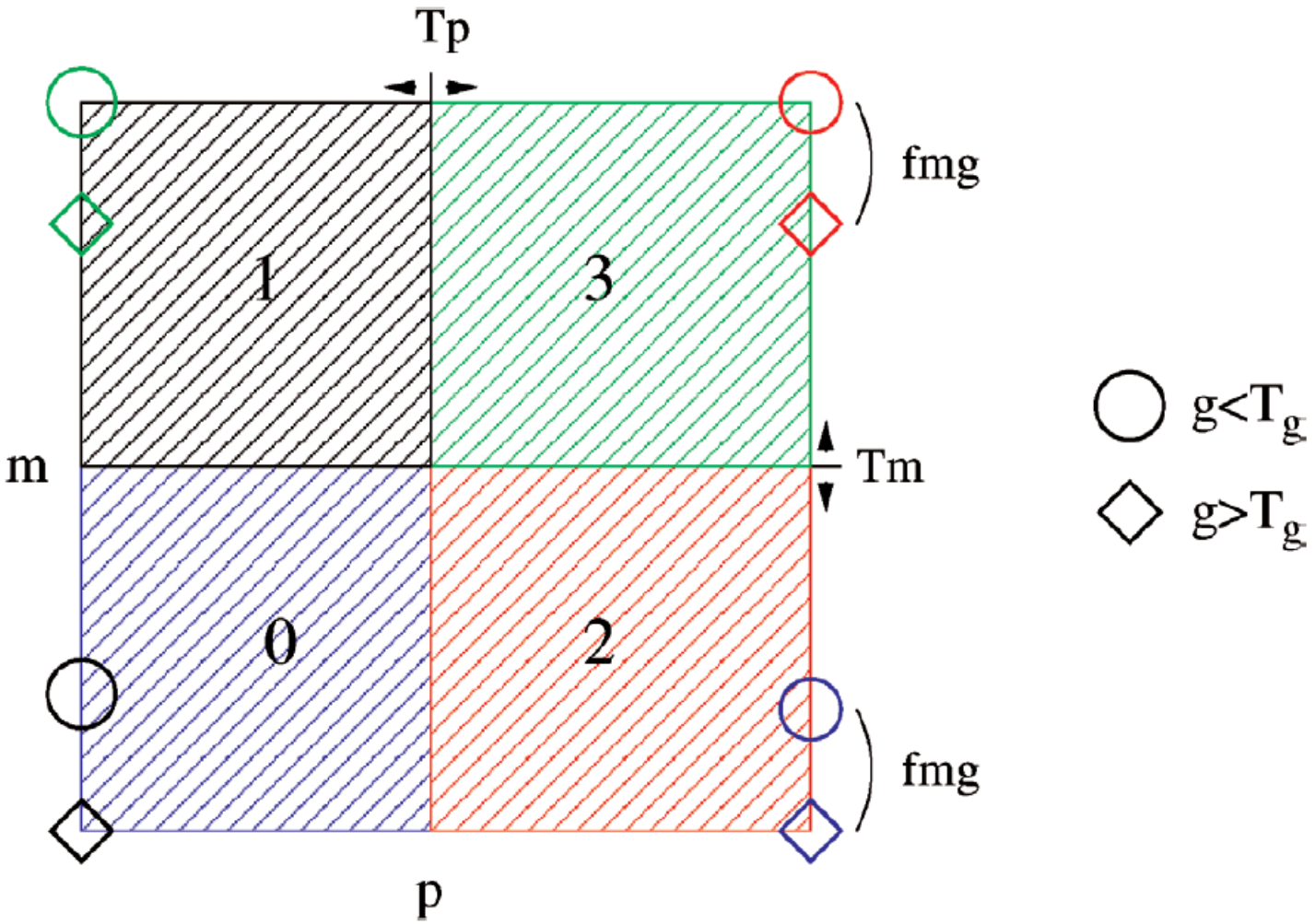
The p-m system: a controlled negative circuit



$$fmp = \frac{W_{mp}}{1 - a_m}; \qquad fmg = \frac{W_{mg}}{1 - a_m}$$

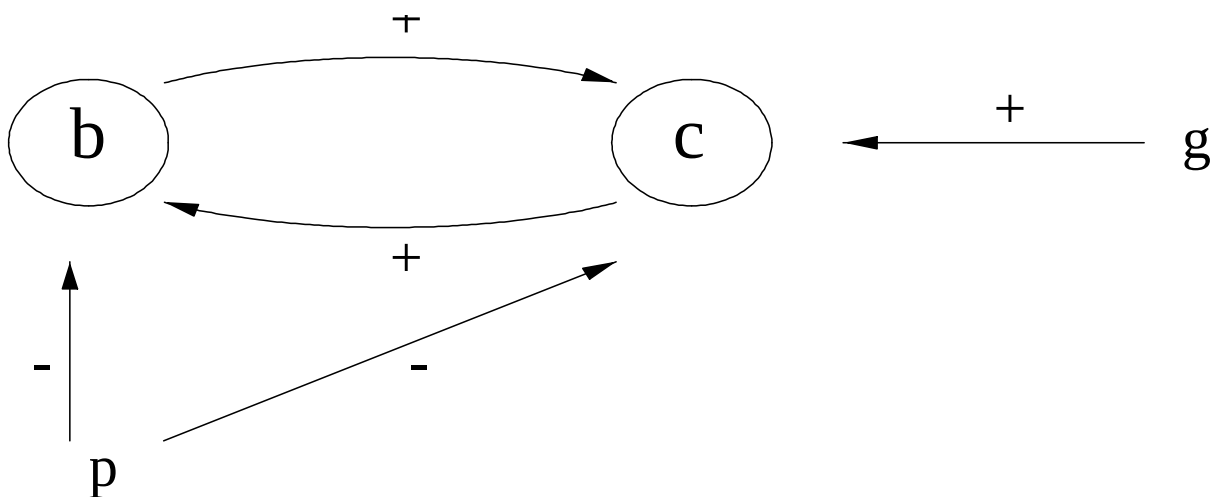
The p-m system: a controlled negative circuit

	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	$0 \to 2$ $\uparrow \quad \downarrow$ $1 \leftarrow 3$

The p-m system: a controlled negative circuit

- The period of the oscillations will depend on the 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*.
- 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 $T_m > fmp$.

The c-b system: a doubly controlled positive circuit

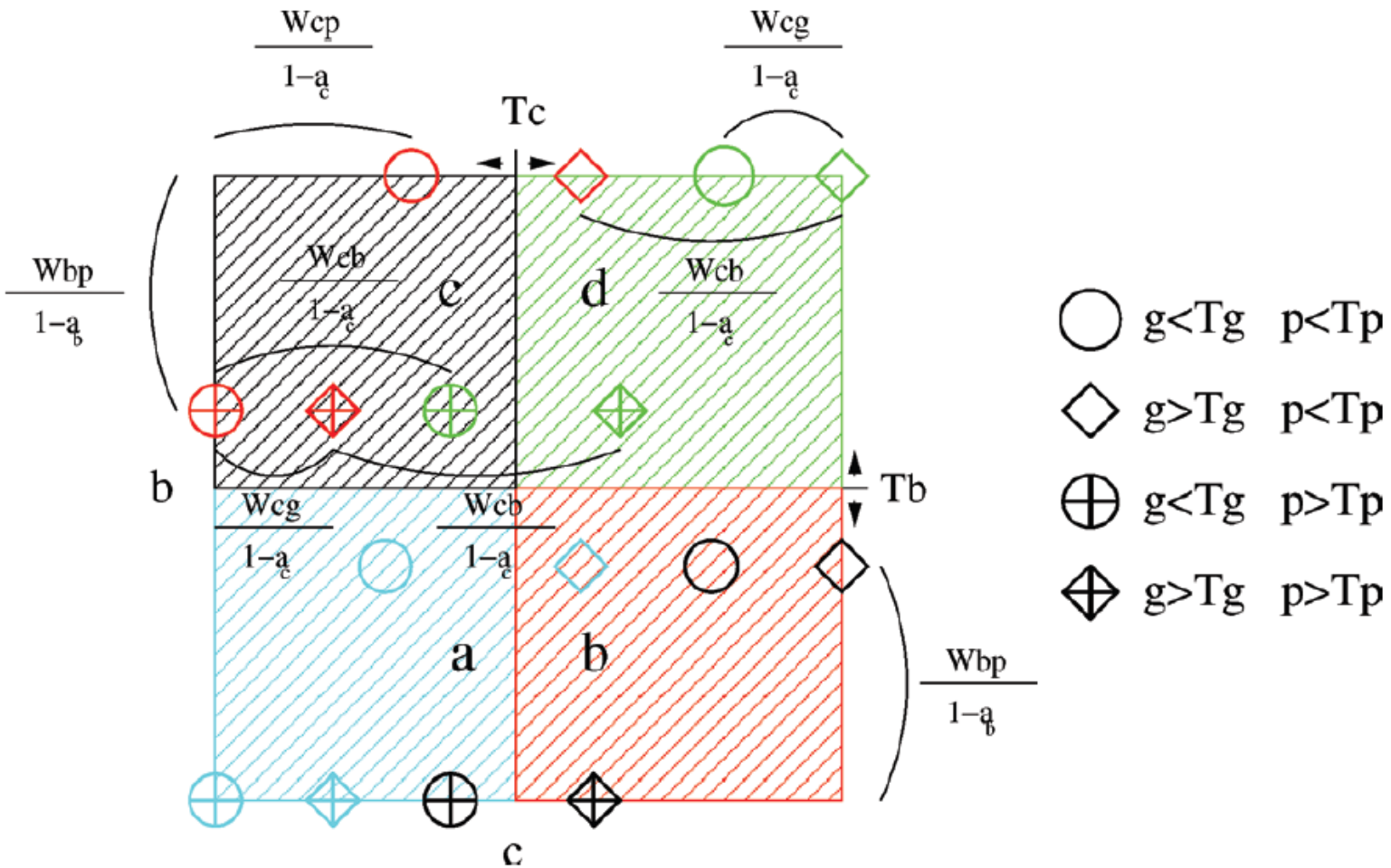


$$
\begin{aligned}
b(t+1) &= a_b b(t) + W_{bc} H(c(t) - T_c) + W_{bp} H(T_p - p(t)) \\
c(t+1) &= a_c c(t) + W_{cb} H(b(t) - T_b) + W_{cp} H(T_p - p(t)) \\
&\quad + W_{cg} H(g - T_g)
\end{aligned}
$$

$$
fcp = \frac{W_{cp}}{1-a_c}; \qquad fcb = \frac{W_{cb}}{1-a_c}; \qquad fcg = \frac{W_{cg}}{1-a_c}; \qquad (fcp + fcb + fcg = 1)
$$

$$
fbc = \frac{W_{bc}}{1-a_b}; \qquad fbp = \frac{W_{bp}}{1-a_b}; \qquad (fbc + fbp = 1)
$$

The c-b system: a doubly controlled positive circuit



The c-b system: a doubly controlled positive circuit

TABLE 2A. (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→d←b ↑ c
$T_c < fcp$ $T_b > fbp$	$b \to 1$ $c \to fcp + fcb$	a→b→d ↑ c
$fcp + fcb > T_c > fcp$ $T_b < fbp$	$b \to 1$ $c \to fcp + fcb$	a→c→d ↑ 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↻ a↻ b⇆c
$T_c > fcp + fcb$ $T_b < fbp$	$b \to fbp$ $c \to fcp + fcb$	a→c←b ↑ d
$T_c > fcp + fcb$ $T_b > fbp$	$b \to fbp$ $c \to fcp$	b→c→a ↑ d

The c-b system: a doubly controlled positive circuit

TABLE 2B. (Case $g < T_g$ and $p > T_p$)

$g < T_g$, $p > T_p$	Asympt. behavior	Coding
$T_c < fcb$ $T_b < fbc$	$b \longrightarrow 0, c \longrightarrow 0$ or $b \longrightarrow fbc, c \longrightarrow fcb$ or oscillation	a↻ d↻ b⇆c
$T_c < fcb$ $T_b > fbc$	$b \longrightarrow 0$ $c \longrightarrow 0$	c→b→a ↑ d
$T_c > fcb$ $T_b < fbc$	$b \longrightarrow 0$ $c \longrightarrow 0$	b→c→a ↑ d
$T_c > fcb$ $T_b > fbc$	$b \longrightarrow 0$ $c \longrightarrow 0$	c→a←b ↑ d

The c-b system: a doubly controlled positive circuit

TABLE 2C. (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

The c-b system: a doubly controlled positive circuit

TABLE 2D. (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

Biological implications and the coupled system

- Biological parameter domain:
  - In the absence of cell abnormalities or oncogenes ($g < T_g$), normal p53 will be at a low level.

$$T_m < fmg$$

  - On the absence of oncogenes and low p53, cell growth should not be explosive

$$T_c > fcp + fcb$$
$$T_b > fbp$$

  then also $T_c > fcb$.
- If $g < T_g$ and $p > T_p$, then $b \longrightarrow 0$ and $c \longrightarrow 0$. That is, in the absence of oncogenes, expression of p53 blocks cell growth.
- In all cases, when $g > T_g$ (presence of oncogenes) and $p < T_p$, there is explosive cell growth ($c \longrightarrow 1$). Only in the special case $T_c > fcg + fcp$ and $T_b > fbp$ there are other solutions, but even in this case $c \longrightarrow 1$ is a possible solution.

Biological implications and the coupled system

- The most interesting situation for cancer control is to analyze the effect of expressed p53 ($p > T_p$) in the presence of oncogenes ($g > T_g$) - (Table 2D)
- For $T_c > fcg + fcb$ (and any T_b) p53 controls cell growth, keeping it at a low level ($c \longrightarrow fcg$). A large value of T_c relates to the effectiveness of cell growth to stimulate the an increased level of blood supply. The larger T_c the less effective is the stimulation signal.
- For T_c values smaller than $fcg + fcb$ the inhibitory effect of p53 may not be so effective. 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.

Biological implications and the coupled system

An example: ($T_m < fmg$, $T_c > fcp + fcb$ and $T_b > fbp$).
The following figures show the dynamical evolution of the variables p, m, b, and c for the parameter values

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

and

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

This corresponds to the situation

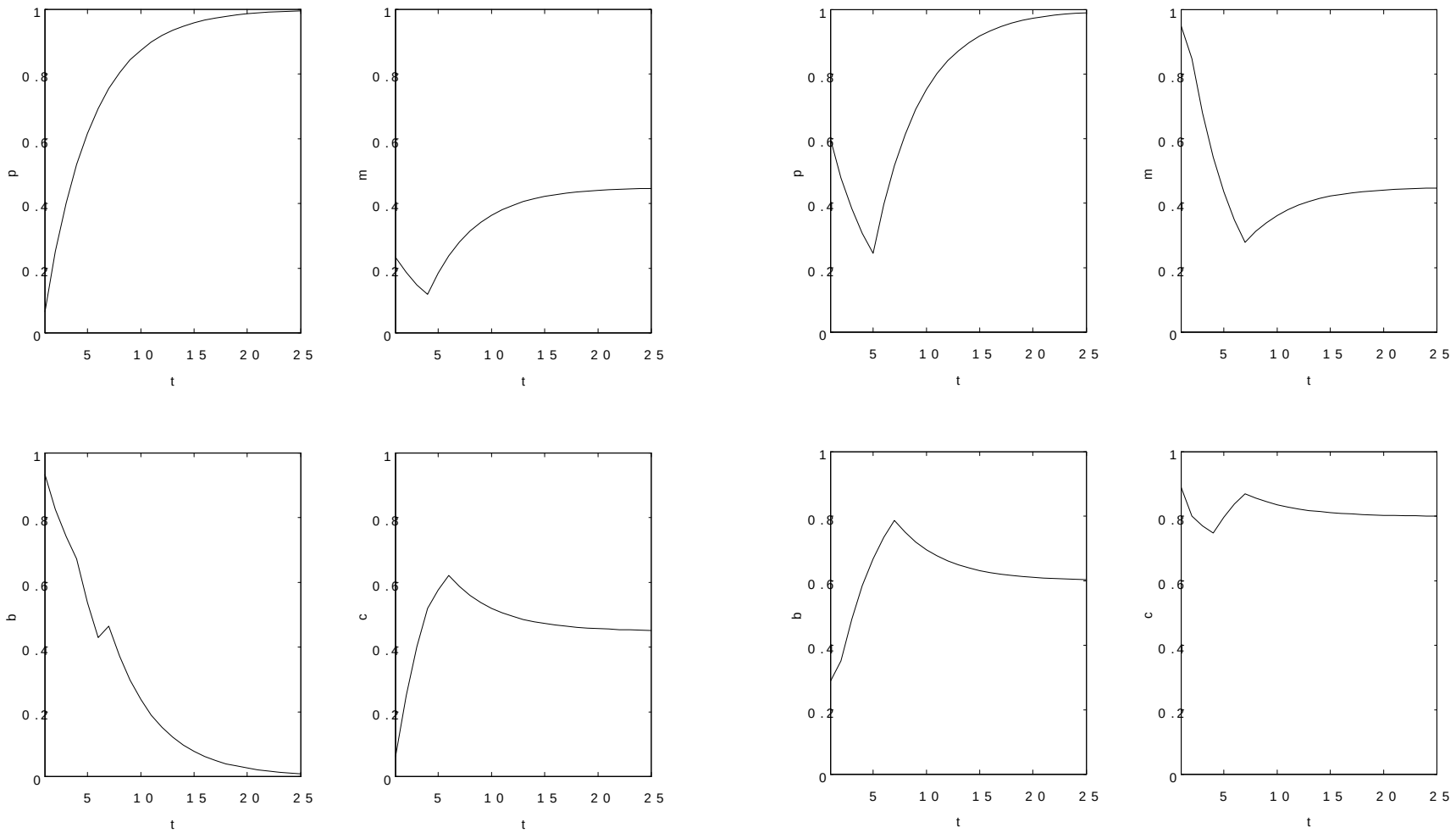
$$T_m < fmg,\ T_m > fmp$$

in the $p - m$ system and

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

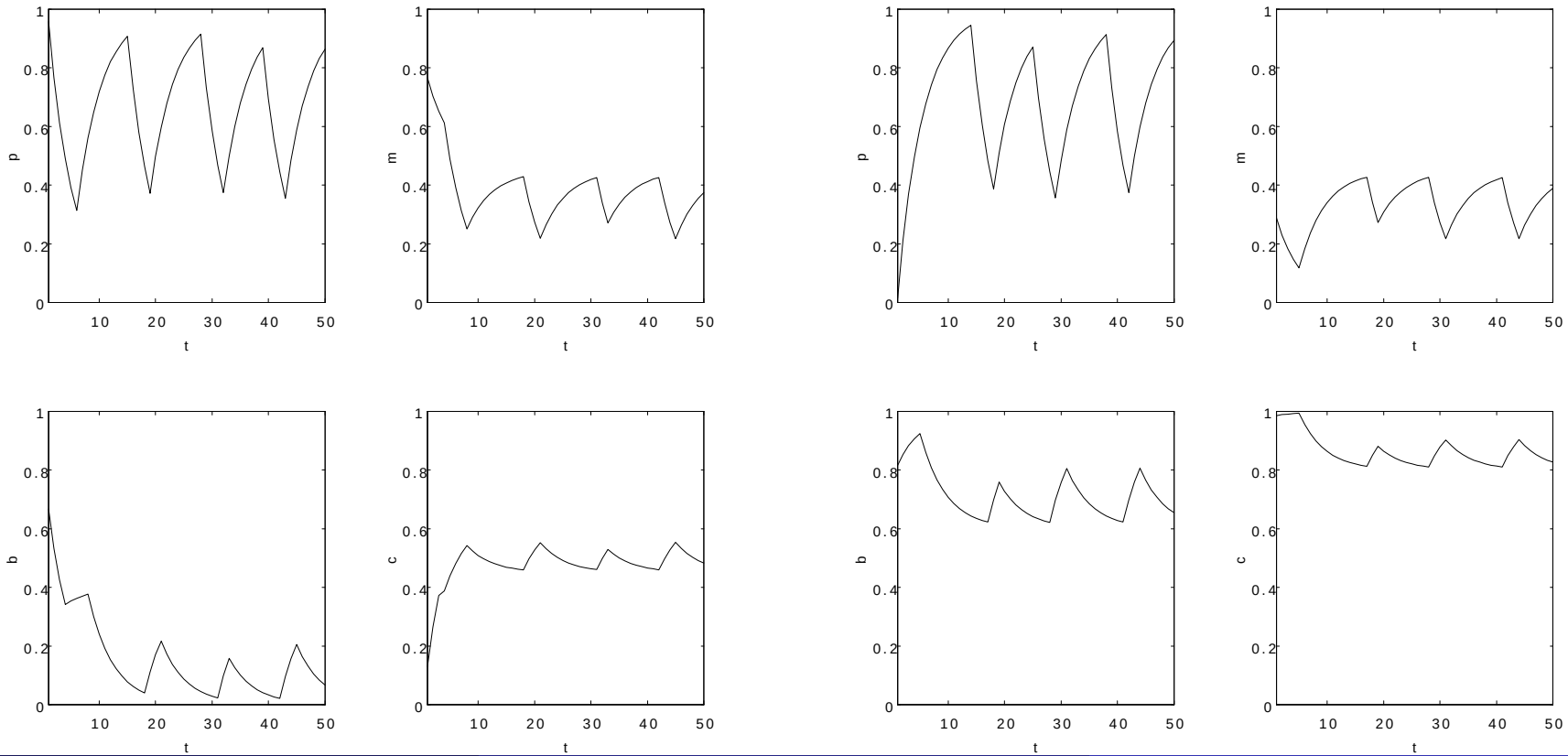
in the $c - b$ system.
The parameter values are the same for both figures. One sees that depending on the initial conditions, in one case the p53 action keeps c at a low level and in the other it does not.

Biological implications and the coupled system



Biological implications and the coupled system

The same parameters for the $c - b$ system, but $T_m = 0.4$, that is, $T_m < fmg$ and $T_m < fmp$. Oscillation in the $p - m$ system but the action on the $c - b$ system is similar and it again depends on the initial conditions.

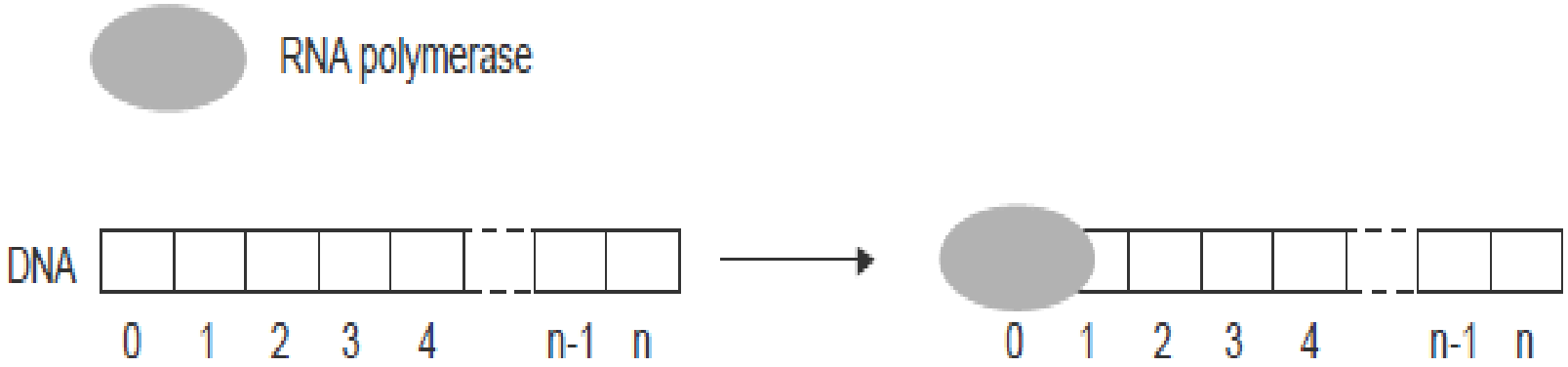


Conclusions

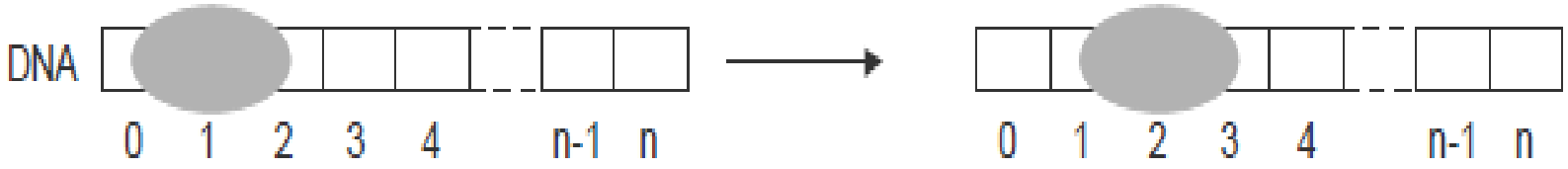
1 - A complete mathematical characterization of its dynamical solutions and how they depend on the parameter values (thresholds and couplings). Threshold positions corresponds to stimulation or inhibition of the mechanisms of gene expression. A guide for therapeutic action.
2 - There is a biologically reasonable region of parameters for which the successful action of p, as an inhibitor of cell growth, strongly depends on the initial conditions.
3 - 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 loss of genetically normal p53 action: *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.*
4 - The model suggests there is a range of biological parameters for which there would be a purely dynamic explanation for the failure of p53.

A stochastic reformulation ?

Gene expression is the result of a large number of discrete events



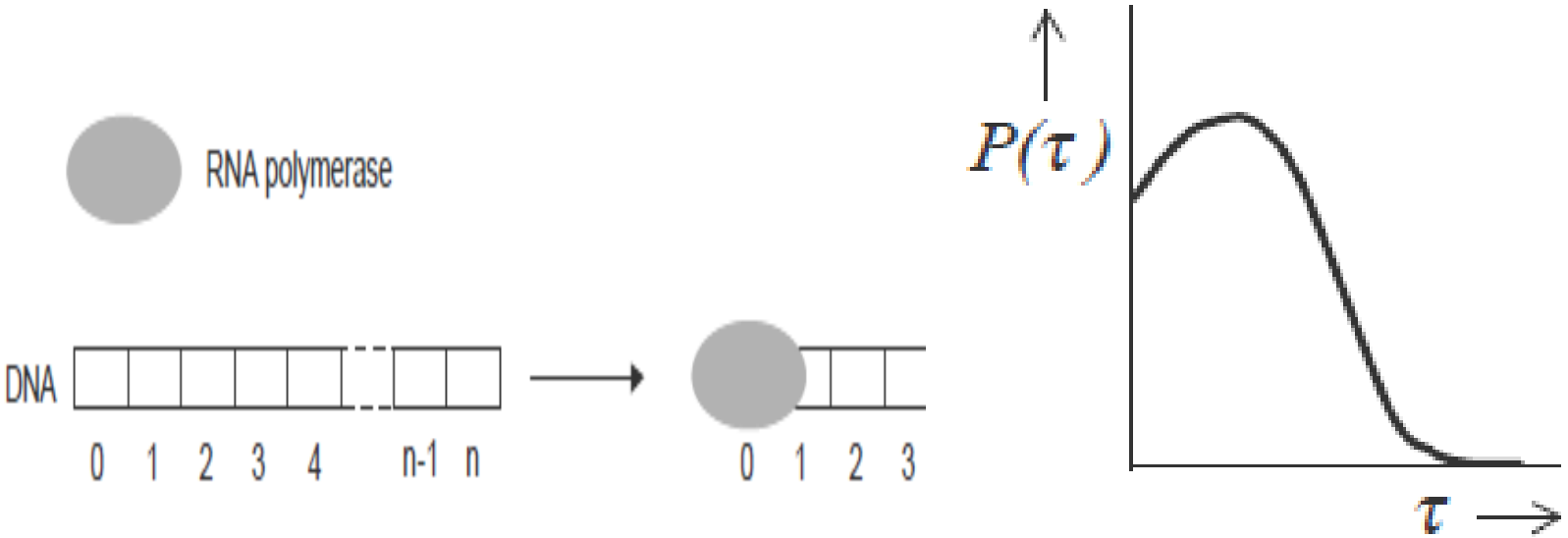
$$DNA + RNAP \rightarrow DNA_0 \bullet RNAP$$



$$DNA_i \bullet RNAP \rightarrow DNA_{i+1} \bullet RNAP$$

A stochastic reformulation ?

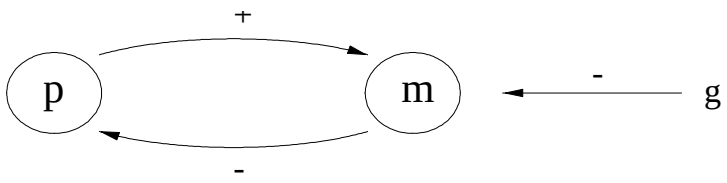
Therefore, gene expression is a stochastic process with random time intervals between the reactions



A stochastic reformulation ?

Thresholds and delays are essential features in biological networks.
A stochastic model should mantain thresholds and represent delays by a stochastic time. May be represented by density-dependent transition probabilities.

The controlled p-m system



$$\begin{aligned} p(t+\Delta t) &= a_p p(t) + W_{pm} H(T_m - m(t)) B_1 \\ m(t+\Delta t) &= a_m m(t) + W_{mp} H(p(t) - T_p) B_2 \\ &\quad + W_{mg} H(T_g - g) B_2 \end{aligned}$$

$B_i = 1$ with probability p and $= 0$ with probability $1-p \Longrightarrow < \tau > = \frac{\Delta t}{p}$

References

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