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Time delay as a key to apoptosis induction in the p53 network

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Abstract. A feedback mechanism that involves the proteins p53 and mdm2, induces cell death as a controlled response to severe DNA damage. A minimal model for this mechanism demonstrates that the response may be dynamic and connected with the time needed to translate the mdm2 protein. The response takes place if the dissociation constant k between p53 and mdm2 varies from its normal value. Although it is widely believed that it is an increase in k that triggers the response, we show that the experimental behaviour is better described by a decrease in the dissociation constant. The response is quite robust upon changes in the parameters of the system, as required by any control mechanism, except for few weak points, which could be connected with the onset of cancer.

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1 Introduction

In healthy cells, a loopback mechanism involving the protein p53 is believed to cause growth arrest and apoptosis as a response to DNA damage [1–4]. Mutations in the sequence of p53 that potentially interfere with this mechanism have been observed to lead to the upraise of cancer [5,6].

Under normal conditions the amount of p53 protein in the cell is kept low by a genetic network built of the mdm2 gene, the mdm2 protein and the p53 protein itself. p53 is produced at a essentially constant rate and promotes the expression of the mdm2 gene [7]. On the other hand, the mdm2 protein binds to p53 and promotes its degradation [8], decreasing its concentration. When DNA is damaged, a cascade of events causes phosphorylation of several serines in the p53 protein, which modifies its binding properties to mdm2 [9]. As a consequence, the cell experiences a sudden increase in the concentration of p53, which activates a group of genes (*e.g.*, p21, bax [10]) responsible for cell cycle arrest and apoptosis. This increase in p53 can reach values of the order of 16 times the basal concentration [11].

A qualitative study of the time dependence of the concentration of p53 and mdm2 has been carried out in reference [7]. Approximately one hour after the stress event (*i.e.*, the DNA damage which causes phosphorylation of p53 serines), a peak in the concentration of p53 is observed, lasting for about one hour. This peak partially overlaps with the peak in the concentration of mdm2, lasting from ≈ 1.5 to ≈ 2.5 hours after the stress event. Another small peak in the concentration of p53 is observed after several hours.

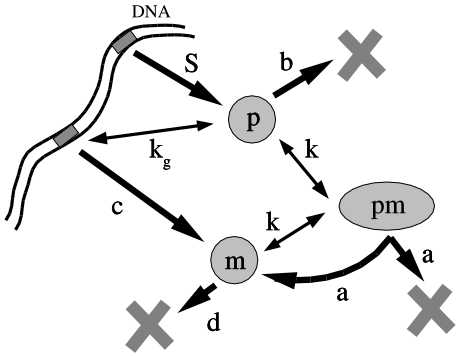

Fig. 1. A sketch of the loopback mechanism which control the amount of p53 in the cell. The grey crosses indicate that the associated molecule leaves the system.

The purpose of the present work is to provide the simpest mathematical model which describes all the known aspect of the p53–mdm2 loop, and to investigate how the loop is robust to small variations to the ingredients of the model. The "weak points" displayed by the system, namely those variations in some parameters which cause abrupt changes in the overall behaviour of the loop, are worth to be investigated experimentally because they can contain informations about how a cell becomes tumoral.

The model we suggest is described in Figure 1. The total number of p53 molecules, produced at constant rate S, is indicated with p. The amount of the complex built

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of p53 bound to mdm2 is called pm. This complex causes the degradation of p53 (through the ubiquitin pathway), at a rate a, while mdm2 re–enters the loop. Furthermore, p53 has a spontaneous decay rate b. The total number of mdm2 proteins is indicated as m. Since p53 activates the expression of the mdm2 gene, the production rate of mdm2 is proportional (with constant c) to the probability that the complex p53/mdm–gene is built. We assume that the complex p53/mdm2–gene is at equilibrium with its components, where k_g is the dissociation constant and only free p53 molecules (whose amount is $p-pm$) can participate into the complex. The protein mdm2 has a decay rate d. The constants b and d describe not only the spontaneous degradation of the proteins, but also their binding to some other part of the cell, not described explicitly by the model. The free proteins p53 and mdm2 are considered to be at equilibrium with their bound complex pm, and the equilibrium constant is called k.

The dynamics of the system can be described by the equations

$$\begin{aligned} \frac{\partial p}{\partial t} &= S - a \cdot pm - b \cdot p \\ \frac{\partial m}{\partial t} &= c\frac{p(t-\tau) - pm(t-\tau)}{k_g + p(t-\tau) - pm(t-\tau)} - d \cdot m \\ pm &= \frac{1}{2}\left((p+m+k) - \sqrt{(p+m+k)^2 - 4p \cdot m}\right). \end{aligned} \tag{1}$$

In the second equation we allow a delay τ in the production of mdm2, due to the fact that the transcription and translation of mdm2 lasts for some time after that p53 has bound to the gene.

The choice of the numeric parameters is somewhat difficult, due to the lack of reliable experimental data. The degradation rate through ubiquitin pathway has been estimated to be $a \approx 3\times10^{-2}$ s^{-1} [12], while the spontaneous degradation of p53 is $\approx 10^{-4}$ s^{-1} [7]. The dissociation constant between p53 and mdm2 is $k \approx 180$ [13] (expressed as number of molecules, assuming for the nucleus a volume of 0.6 μm^3), and the dissociation constant between p53 and the mdm2 gene is $k_g \approx 28$ [14]. In lack of detailed values for the protein production rates, we have used typical values, namely $S = 1$ s^{-1} and $c = 1$ s^{-1}. The degradation rate of mdm2 protein has been chosen of the order of $d = 10^{-2}$ s^{-1} to keep the stationary amount of mdm2 of the order of 10^2.

The behaviour of the above model is independent on the volume in which we assume the reaction takes place. That is, multiplying S, c, k_g and k by the same constant ω gives exactly the same dynamics of the rescaled quantities ωp and ωm. Furthermore, due to the fact that the chosen parameters put the system in the saturated regime, an increase in the producing rates S and c with respect to k_g and k will not affect the response. On the contrary, a decrease of S and c with respect to k_g and k can drive the system into a non–saturated regime, inhibiting the response mechanism.

Table 1. Stationary values p^* and m^* for the amount of p53 and mdm2, respectively, calculated at $\tau = 0$. In the last column the eigenvalues of the linearized (around the fixed points p^*, m^*) dynamical matrix are displayed. The real part of the eigenvalues is always negative and the imaginary part, when different from zero, is lower than the real part, indicating that the stationary values are always stable and the dynamics is overdamped.

k	p^*	m^*	$\lambda_{1;2}$
0.18	47.3	33.6	-0.017 ± 0.013i
1.8	49.5	36.8	$-0.012,\ -0.014$
18	63.9	52.4	$-0.011,\ -0.008$
180	**154.6**	**81.3**	$\mathbf{-0.007 \pm 0.001}$**i**
1800	858.3	96.7	$-0.009,\ -0.0008$
18000	4287	99.3	$-0.009,\ -0.0002$
180000	8632	99.6	$-0.009,\ -0.0001$

2 Results with no delay

In the case that the production of mdm2 can be regarded as instantaneous (no delay, $\tau = 0$), the concentration of p53 is rather insensitive to the change of the dissociation constant k. The stationary values of p and m are found as fixed points of the equations 1 (see Appendix) and in Table 1 we list the stationary values p^* of the amount of p53 molecules for values of k spanning seven orders of magnitude around the basal value $k = 180$. Moreover, transient oscillatory behaviour upon change in the dissociation constant k is not observed. This is supported by the fact that the eigenvalues of the stationary points (listed in Tab. 1) have negative real parts, indicating stable fixed points, and rather small imaginary parts indicating absence of oscillations.

More precisely, the variation Δp of the stationary amount of p53 if the dissociation constant undergoes a change Δk can be estimated, under the approximation that $k_g \ll p$ (*cf.* the Appendix), to be

$$\Delta p = \frac{d(S-bp)}{ack_g(a+b)}\Delta k. \tag{2}$$

The fact that Δp is approximately linear with Δk with a proportionality constant which is at most of the order of 10^{-2} makes this system rather inefficient as response mechanism. Furthermore, it does not agree with the experimental data which show a peak of p53 followed, after several minutes, by a peak in mdm2 [7], and not just a shift of the two concentration to higher values.

To check whether the choice of the system parameters affects the observed behaviour, we have repeated all the calculations varying each parameter of five orders of magnitude around the values used above. The results (listed in Tab. 2 for S and k_g and not shown for the other parameters) indicate the same behaviour as above (negative real part and no or small imaginary part in the eigenvalues). Consequently, the above results about the dynamics

Table 2. Same as in Table 1, varying some of the parameters which define the system of five orders of magnitude. In each cell it is indicated the quantity at $k = 18$, $k = 180$ (basal value) and $k = 1800$.

	p^*	m^*	$\lambda_{1;2}$
$S = 0.01$	1.6	4.6	$-0.02 \pm 0.006i$
	4.6	13.6	$-0.007,\ -0.005$
	15.1	34.6	$-0.009,\ -0.001$
$S = 0.1$	8.3	15.1	$-0.01 \pm 0.008i$
	20.2	37.8	$-0.007 \pm 0.001i$
	81.1	73.6	$-0.009,\ -0.001$
$S = 1$	63.9	52.4	$-0.01 \pm 0.008i$
	154.6	81.3	$-0.007 \pm 0.001i$
	858	96.7	$-0.009,\ -0.0008$
$S = 10$	70019	99.9	$-0.009,\ -10^{-4}$
	70088	99.9	$-0.009,\ -10^{-4}$
	70756	99.9	$-0.009,\ -10^{-4}$
$S = 100$	970000	99.9	$-0.01,\ -10^{-4}$
	970000	99.9	$-0.01,\ -10^{-4}$
	970000	99.9	$-0.01,\ -10^{-4}$
$k_g = 0.28$	42.5	97.0	$-0.02,\ -0.01$
	121.7	99.6	$-0.009,\ -0.006$
	824.1	99.9	$-0.009,\ -0.006$
$k_g = 2.8$	45.5	81.5	$-0.01 \pm 0.003i$
	125.2	97.0	$-0.009,\ -0.006$
	827.3	99.6	$-0.009,\ -0.008$
$k_g = 28$	63.9	52.4	$-0.01 \pm 0.008i$
	154.6	81.3	$-0.007 \pm 0.001i$
	858	96.7	$-0.009,\ -0.0008$
$k_g = 280$	192.7	36.3	$-0.006 \pm 0.0003i$
	331.1	51.6	$-0.008,\ -0.003$
	1104.1	79.3	$-0.009,\ -0.007$
$k_g = 2800$	1214	29.0	$-0.009,\ -0.0006$
	1380	32.5	$-0.009,\ -0.0006$
	2345	45.3	$-0.009,\ -0.0006$

of p53 seem not to be sensitive to the detailed choice of parameters (on the contrary, the amount of mdm2 is quite sensitive).

3 Results with delay

The dynamics changes qualitatively if we introduce a nonzero delay in equations (1). Keeping that the halflife of an RNA molecule is of the order of 1200 s [16], we repeat the calculations with $\tau = 1200$. Equations (1) are solved numerically, starting from the conditions $p(0) = 0$ and $m(0) = 0$ and making use of a variable–step Adams algorithm. After the system has reached its stationary state under basal condition, a stress is introduced (at time $t = 20\,000$ s) by changing instantaneously the dissociation constant k. In Figure 2 we display a case in which the stress multiplies k by a factor 15 (a), a case in which it divides it by a factor 15 (b) and by a factor 5 (c).

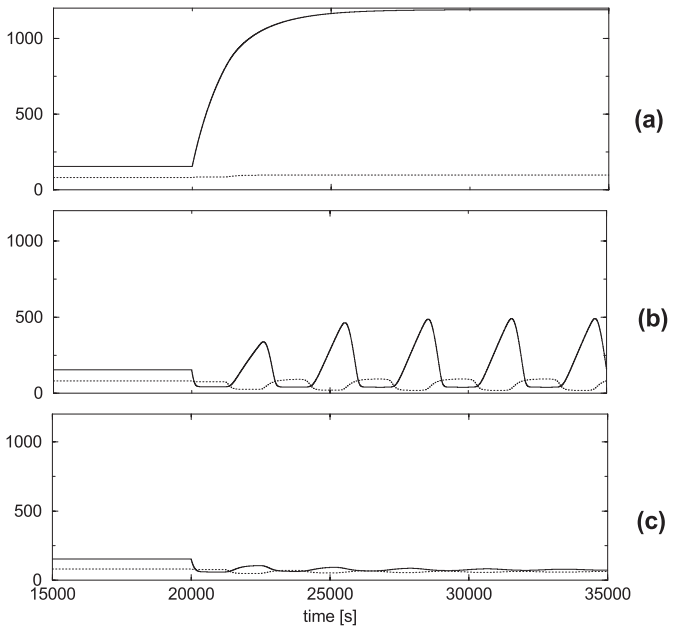

Fig. 2. The response in the concentration of p53 (solid line) and mdm2 (dotted line) upon variation of the dissociation constant k. At time 20 000 s the constant k is multiplied by 15 (a), divided by 15 (b) and divided by 5 (c).

When k is increased by any factor, the response is very similar to the response of the system without delay (*cf. e.g.* Fig. 2a). On the contrary, when k is decreased the system displays an oscillatory behaviour. The relative height $\Delta p/p$ of the response peak is plotted in Figure 3 as a function of the quantity which multiplies k. If the multiplier is larger than 0.1 the response is weak or absent. At the value 0.1 the system has a marked response (*cf.* also Figs. 2b and c). The maximum of the first peak takes place approximately 1200 s after the stress, which is consistent with the lag–time observed in the experiment [7], and the peaks are separated from ≈ 2300 s.

Although it has been suggested that the effect of the stress is to increase the dissociation constant between p53 and mdm2 [6], our results indicate that an efficient response take place if k decreases of a factor ≥ 15 (*cf.* Fig. 2b). One has to notice that the conclusions of reference [6] have been reached from the analysis *in vivo* of the overall change in the concentration of p53, not from the direct measurement of the binding constant after phosphorylation. Our results also agree with the finding that p53asp20 (a mutated form of p53 which mimicks phosphorylated p53, due to the negative charge owned by aspartic acid) binds mdm2 *in vitro* more tightly than p53ala20 (which mimicks unphosphorylated p53) [6].

This hypothesis is supported by molecular energy calculations made with classical force fields. Even if this kind of force fields is not really reliable for the calculation of

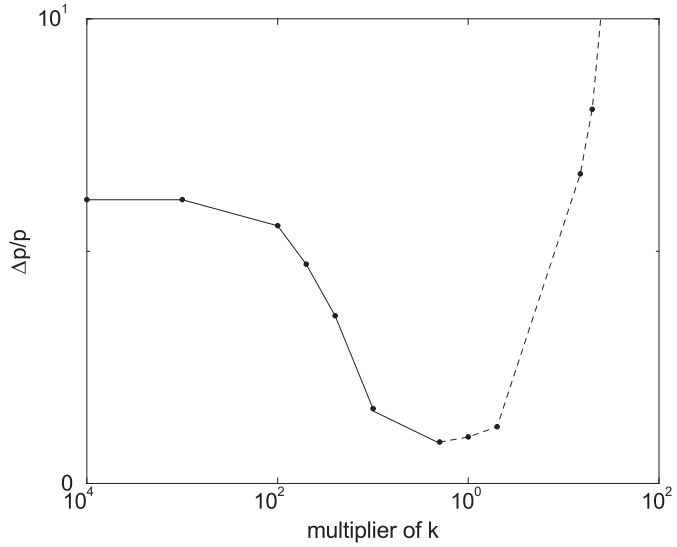

Fig. 3. The relative height of the response peak $\Delta p/p$ with respect to the quantity that multiplies k, mimicking the stress. The dotted line indicates that the system does not display oscillatory behaviour.

binding constants, it gives an estimate of the sign of the change in interaction among p53 and mdm2 upon phosphorylation. We have performed an energy minimization of the conformation of the system composed by the binding sites of p53 and mdm2, starting form the crystallographic positions of reference [13] and using the force fields mm3 [17] and mmff [18], for both the wild–type system and for the system where serine 20 of p53 in phosphorylated. Using mm3 we found that the phosphorylated system has an electrostatic energy 16 kcal/mol lower than the wild–type system, while this difference is 26 kcal/mol using the mmff force field. Our calculations suggest that phopshprylated p53 is more attracted by mdm2 due to the enhanced interaction of phosphorylated SER20 with LYS60, LYS47 and LYS90 of mdm2, and consequently the dissociation constant is lowered.

The robustness of the response mechanism with respect to the parameters of the system, which is typical of many biological systems (*cf.*, *e.g.*, [19,20]), has been checked both to assess the validity of the model and to search for weak points which could be responsible for the upraise of the disease. Each parameter has been varied of five orders of magnitude around its basal quantity. The results are listed in Table 3. One can notice that the response mechanism is quite robust to changes in the parameters a, b and c. For low values of a or c the system no longer oscillates, but displays, in any case, a rapid increase in the amount of p which can kill the cell. This is true also for large values of d. What is dangerous for the cell is a decrease of d or of k_g, which would drop the amount of p53 and let the damaged cell survive. This corresponds either to an increase of the affinity between p53 and the mdm2 gene, or to an increase of mdm2 half–life.

To be noted that, unlike the case $\tau = 0$, the system with delay never displays damped oscillations as a consequence of the variation of the parameters in the range studied in the present work. This sharp behaviour further testifies to the robustness of the response mechanism. Anyway, one has to keep in mind that the oscillating response produces the death of the cell, and consequently the long–time behaviour is only of theoretical interest.

Table 3. The value of $\Delta p/p$ when the parameters a, b, c, d and k_g are scaled of the quantity listed in the first column. (1) indicates that the system does not oscillate and p reaches a stationary value much larger than before the stress. (2) indicates that the system does not display any response to the stress or displays a negative response. The star indicates that the peak appears after 4000 s (for $c = 10^4$) and 25 000 s (for $c = 10^5$).

scale	a	b	c	d	k_g
10^{-2}	(1)	3.43	(1)	(2)	(2)
10^{-1}	(1)	3.46	(1)	(2)	(2)
1	3.2	3.2	3.2	3.2	3.2
10	11.1	2.14	9.2*	(1)	3.2
100	2.1	(2)	1.4*	(1)	(2)

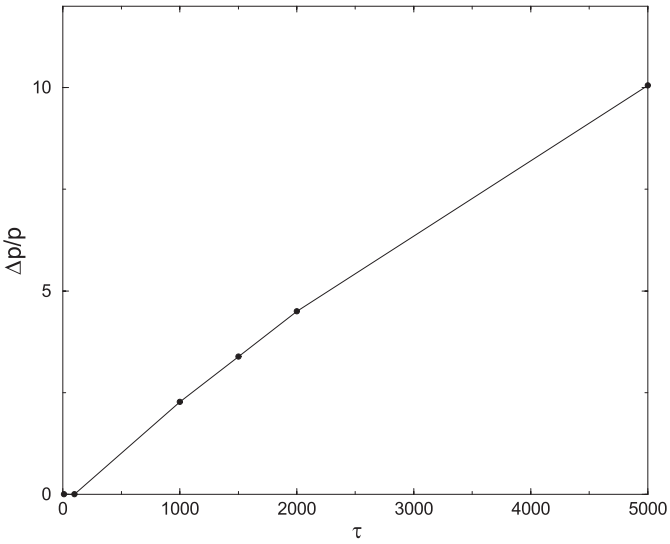

Fig. 4. The dependence of the relative height of the response peak $\Delta p/p$ on the delay τ.

The minimum value of the delay which gives rise to the oscillatory behaviour is $\tau \approx 100$ s. For values of the delay larger than this threshold, the amplitude of the response is linear with τ (*cf.* Fig. 4), a fact which is compatible with the explanation of the response mechanism of Section 4.

The lag time before the p53 response is around 3000 s (in accordance with the 1h delay observed experimentally [7] and is independent on all parameters, except c and τ. The dependence of the lag time on τ is approximately linear up to 5000 s (the longest delay analyzed). Increasing c the lag time increases to 8000 s (for $c = 10^4$) and 25 000 s (for $c = 10^5$). On the other hand, the period of oscillation depends only on the delay τ, being approximately linear with it.

We have repeated the calculations squaring the variable p in the second of equations (1), to keep into account the cooperativity induced by the fact that the active form of p53 is a dimer of dimers [21]. The results display qualitative differences neither for non–delayed nor for the delayed system.

4 Discussion

All these facts can be rationalized by analyzing the mechanism which produces the response. The possibility to trigger a *rise* in p53 as a dynamic response to an *increased* binding between p53 and mdm2, relies on the fact that a sudden increase in p–m binding diminishes the production of mdm2, and therefore (subsequently) diminishes the amount of pm. In other words, while the change in k has no direct effect in the first of equations (1), it directly reduces mdm2 production by subtracting p53 from the gene which produces mdm2.

Mathematically, the oscillations arise because the saturated nature of the binding pm imply that pm is approximately equal to the minimum between p and m. Each time the curves associated with p and m cross each other (either at a given time or τ instants before), the system has to follow a different set of dynamic equations than before, finding itself in a state far from stationarity. This gives rise to the observed peaks.

To be precise, the starting condition (before the stress) is $m < p$. The stress reduces the dissociation constant k of, at least, one order of magnitude, causing a drop in p, which falls below m. For small values of k (to be precise, for $k \ll \min(|p-m|, p, m)$), one can make the simplification $pm \approx \min(p, m)$, and consequently rewrite equations (1) as

$$\text{for } p < m \quad \frac{\partial p}{\partial t} = S - (a+b)p \tag{3}$$

$$\text{for } p(t-\tau) < m(t-\tau) \quad \frac{\partial m}{\partial t} = -dm \tag{4}$$

$$\text{for } p > m \quad \frac{\partial p}{\partial t} = S - am - bp \tag{5}$$

$$\text{for } p(t-\tau) > m(t-\tau) \quad \frac{\partial m}{\partial t} = c\frac{p(t-\tau) - m(t-\tau)}{k_g + p(t-\tau) - m(t-\tau)} - dm. \tag{6}$$

Each period after the stress can be divided in four phases. In the first one $p < m$ and $p(t-\tau) < m(t-\tau)$, so that p stays constant at its stationary value $S/(a+b) \approx p^*$, while m decreases with time constant d^{-1} towards zero (not exactly zero, since the approximated Equations do not hold for $m \sim k$). In the second phase one has to consider the second ($p(t-\tau) < m(t-\tau)$) and the third ($p > m$) equations (4, 5). The new stationary value for p is $(S - am)/b \approx S/b$ which is much larger than p^* since $b \ll a$. This boost takes place in a time of the order of b^{-1}, so if $b^{-1} > \tau$, as in the present case, p has no time to reach the stationary state and ends in a lower value. In the meanwhile, m remains in the low value given by equation (4). At a time τ after the stress equation (4) gives way to equation (6). The latter is composed by a positive term which is $\approx c$ if $p(t-\tau) - m(t-\tau) \gg k_g$ and ≈ 0 under the opposite condition. Since $p(t-\tau) \gg m(t-\tau)$ (it refers to the boost of p), then the new stationary value of m is $c/d \approx m^*$. The raise of m takes place in a time of the order of d^{-1} and causes the decrease of p, whose production rate is ruled by $S - am$. The fourth phase begins when p approaches m. Now one has to keep equations (3, 6), so that p returns to the basal value p^*, while m stays for a period of τ at the value $c/d \approx m^*$ reached in the third phase. After such period, equation (4) substitutes equation (6) and another peak takes place.

The height of the p53 peak is given by S/b if p has time to reach its stationary state of phase two (*i.e.*, if $b^{-1} < \tau$), or by $S/b(1 - \exp(-b\tau))$ if the passage to the third phase takes place before it can reach the stationary state. The width of the peak is $\approx \tau$ and the spacing among the peaks $\approx \tau$, so that the oscillation period is $\approx 2\tau$.

The necessary conditions for the response mechanism to be effective are 1) that $s/a \ll c/d$, that is that the stationary value of p just after the stress is much lower than the stationary value of m, 2) that $b \ll a$, in such a way that the stationary state of p in the second phase is much larger than that in the first phase, in order to display the boost, 3) that $d^{-1} < \tau$, otherwise m has not enough time to decrease in phase one and to increase in phase three.

The failure of the response for low values of a (*cf.* Tab. 3) is due to the fall of condition 2), the failure for small c is caused by condition 1), the failure at small and large values of d is associated with conditions 3) and 1), respectively. At low values of k_g the response does not take place because the positive term in equation (6) is always $\sim c$, and thus m never decreases.

5 Conclusions

To sum up, we have shown that the delay is an essential ingredient of the system to have a ready and robust peak in p53 concentration as response to a damage stress. In order to have a peak which is comparable with those observed experimentally, the dissociation constant between p53 and mdm2 has to decrease of a factor 15. Although it is widely believed that phosphorylation of p53 increases the dissociation constant, we observe an oscillating behaviour similar to the experimental one only if k decreases. In this case the response is quite robust with respect to the parameters, except upon increasing of the half–life of mdm2 and upon decreasing of the dissociation constant between p53 and the mdm2 gene, in which cases there is no response to the stress. Moreover, an increase in the production rate of mdm2 can delay the response and this can be dangerous to the cell as well. We hope that detailed experimental measurements of the physical parameters of the system will be made soon, in order to improve the model and to be able to make more precise predictions about the weak point of the mechanism, weak points which could be intimately connected with the upraise of cancer.

Appendix

The stationary condition for equations (1) without delay can be found by the intersection of the curves

$$m(p) = \frac{c(a+b)p - cs}{d(a+b)p - d(S - ak_g} \tag{7}$$

$$m_k(p) = \frac{(S-bp)((a+b)p + ak - S)}{a((a+b)p - S)}, \tag{8}$$

which have been obtained by the conditions $\dot{p} = \dot{m} = 0$, explicitating pm from the first of equations (1) and substituting it in the second and the third, respectively. To be noted that m_k is linear in k.

The variation Δp of the stationary value of p53 upon change Δk in the dissociation constant can be found keeping that

$$\frac{\mathrm{d}p}{\mathrm{d}k} = \frac{\mathrm{d}p}{\mathrm{d}m}\frac{\mathrm{d}m_k}{\mathrm{d}k} \approx \frac{d(S-bp)}{ck_g(a+b)}, \tag{9}$$

where the approximation $k_g \ll p$ has been used. Consequently,

$$\Delta p = \frac{d(S-bp)}{ck_g(a+b)}\Delta k, \tag{10}$$

which assumes the largest value when p is smallest. Using the parameters listed above, the proportionality constant is, at most, 10^{-2}.

Furthermore, keeping that $pm < \min(p, m)$ for any value of p and m, the eigenvalues of the dynamical matrix have negative real part, indicating that the stationary points are always stable.

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