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A MESOSCOPIC APPROACH TO MODEL REGULATION OF APOPTOSIS BY P53

UNA APROXIMACION MESOSC'OPICA PARA MODELAR LA REGULACION DE LA APOPTOSIS POR p53.

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A mechanism to describe the apoptosis process at mesoscopic level through p53 is proposed in this paper. A deterministic model given by three differential equations is deduced from the mesoscopic approach, which exhibits sustained oscillations caused by a supercritical Andronov-Hopf bifurcation. Taking as hypothesis that the p53 sustained oscillation is the fundamental mechanism for apoptosis regulation; the model predicts that it is necessary a strict control of p53 to stimulated it, which is an important consideration to established new therapy strategy to fight cancer.

Se propone un mecanismo para describir a nivel mesoscópico el proceso de apoptosis a través de la acción de la proteína p53. A partir del modelo mesoscópico se obtiene un modelo determinista de tres ecuaciones diferenciales que exhibe la aparición de oscilaciones sostenidas en el nivel de p53, producto de una bifurcación supercrítica de Andronov-Hopf. Tomando como base la hipótesis de que estas oscilaciones constituyen el mecanismo fundamental para la regulación del proceso de apoptosis, el modelo predice que es necesario un estricto control de la p53 para estimular este, lo cual constituye una consideración importante para el establecimiento de nuevas terapias contra el cáncer.

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INTRODUCTION

Three decades of p53 research have led to many advances in understanding the function of p53 in relation to longevity and aging [1], metabolism regulation [2], tumour suppression and apoptosis process, which are important aspects because many human cancers show resistance to apoptosis [3,4]. The apoptosis process consists in the programmed cellular death which occurs when DNA damage is detected, but can not be repaired. In this scenario, the cell may reproduce in a mutated form, later appearing as cancer.

Experimental studies of p53 and Mdm2 behaviour in response to DNA damage show damped oscillation of p53 concentration at cell population level and undamped oscillation of p53 in single cells [5,6,7,8]. Although the oscillatory behaviour is ubiquitous for biological systems [9,10,11], the significance of p53 oscillations still remains unclear [8].

Taking into account that mathematical models represent a manner for formalizing the knowledge of living systems used in theoretical biology, deterministic and stochastic models have been proposed to describe this behaviour, which generally take the p53-Mdm2 negative feedback loop as the key mechanism that determines the p53 oscillations [7,12,13,14,15,16,17].

In this work, we propose a mechanism for the apoptosis regulation by p53 sustained oscillations. This mechanism was developed taking into account the experimental results reported being related with the oscillation in the amount of p53 that is present in the damaged cells [5,6,7,8] and the role of oscillation in the biological system. A mesoscopic approach to be used, to establish cancer´s therapeutic strategies [18,19] is obtained from the proposed mechanism.

APOPTOSIS REGULATION MECHANISM AND MESOSCOPIC MODEL

To obtain a mechanism to predict the dynamics of p53 at cellular level associated to the apoptosis process, the following considerations were made: 1) the p53 activation is stimulated by a virtual species Dm, which is associated with the DNA damage level; 2) p53 stimulates the synthesis of Mdm2; 3) Mdm2 stimulates the p53 degradation and 4) the level of Dm decreases with the increased p53 level. The increase in p53 retards the mitosis processes while the damage is repaired [8]. This proposed mechanism is shown in Figure 1.

To describe the system at microscopic level, n was considered as state vector, whose components are associated with the total number of each species contained in a region of volume Ω::

$$\overline{n} = \begin{bmatrix} P_{53} \\ Mdm2 \\ D_m \end{bmatrix}, \qquad (1)$$

while at macroscopic level was considered that the components of the state vector c are the number of each species per unit of volume:

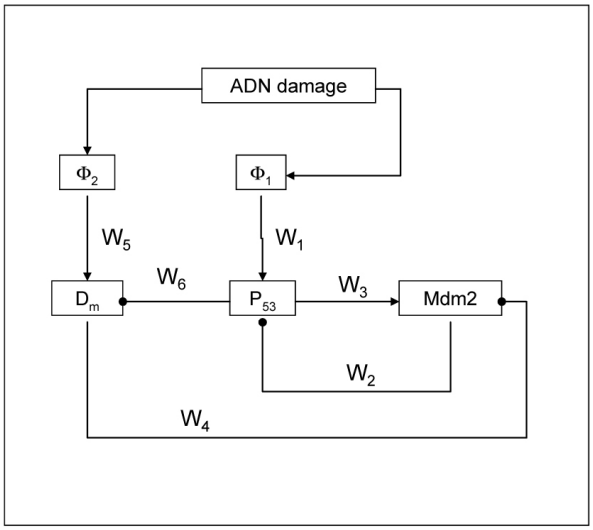
$$\overline{c} = \begin{bmatrix} x \\ y \\ z \end{bmatrix}, \qquad (2)$$

in such way that the relation between both is given by:

$$\overline{n} = \Omega \overline{c}. \qquad (3)$$

We assumed that each *p* processes at microscopic level occurs with a transition probability per unit time W_p, which must be supposed *a priori*. So, the following facts were established: 1) the synthesis of p53 via ADN has a transition probability given by $W_1 = \Phi_1 \Omega$, where Φ_1 is the p53 basal synthesis rate constant; 2) the degradation of p53 because of the Mdm2 action, $W_2 = K.\Phi^{-1}.p53.Mdm_2$, where K is the p53 degradation rate constant; 3) The synthesis of Mdm_2 is stimulated by p53, $W_3 = A.P53$, where A is the Mdm_2 synthesis rate constant; 4) the inhibition of Mdm_2, $W_4 = B.\ \Omega^{-1}.Mdm_2.D_m$, where B is the Mdm_2 inhibition rate constant; 5) the synthesis of D_m is stimulated by the damage magnitude, $W_5 = \Phi 2\Omega$, where Φ 2 is a rate constant associated to the damage level and 6) the damage inhibition caused by the p53 action is, $W_6 = C.P53$, where C is a rate constant associated to damage reparation because of the p53 action. The transition probabilities per unit of time are shown in Figure 1.

From the transition probability per unit time established a *priori*, the obtained Fokker-Planck equation (FPE) [18,19], expressed as a function of the macroscopic variables, is written as:



$$\begin{aligned} \frac{\partial P(x,y,z;t)}{\partial t} = & -\frac{\partial}{\partial x}(\Phi_1 - Kxy)P(x,y,z;t) \\ & -\frac{\partial}{\partial y}(Ax - Byz)P(x,y,z;t) \\ & -\frac{\partial}{\partial z}(\Phi_2 - Cx)P(x,y,z;t) \\ & +\frac{1}{2}\frac{\partial^2}{\partial x^2}\frac{1}{\Omega}(\Phi_1 + Kxy)P(x,y,z;t) \\ & +\frac{1}{2}\frac{\partial^2}{\partial y^2}\frac{1}{\Omega}(Ax + Byz)P(x,y,z;t) \\ & +\frac{1}{2}\frac{\partial^2}{\partial z^2}\frac{1}{\Omega}(\Phi_2 + Cx)P(x,y,z;t) \end{aligned} \qquad (4)$$

$$P(x_0, y_0, z_0; 0) = 1.$$

The FPE (4) describes the temporal behaviour of the system through the probability P(x,y,z;t) , but it does not have an exact solution because of the nonlinear terms, associated with the transition probability per unit time established a priori. So, it is necessary to apply an analytical approximated method, from which we can obtain the temporal behaviour of the expected values and the covariance matrix. In this case we only arrive to a partial description of the system, but it is sufficient to determine the behaviour of the internal fluctuations and their relation with the behaviour of the expected values. The first moment of the transition probabilities per unit time, which is on the right side of the FPE associated to the first partial derivatives, is written as:

$$\overline{\alpha} = \begin{bmatrix} \Phi_1 - Kxy \\ Ax - Byz \\ \Phi_2 - Cx \end{bmatrix} \qquad (5)$$

whereas the second moment associated to the second partial derivatives is:

$$\hat{\beta} = \frac{1}{\Omega}\begin{bmatrix} \Phi_1 + Kxy & 0 & 0 \\ 0 & Ax + Byz & 0 \\ 0 & 0 & \Phi_2 + Cx \end{bmatrix}. \qquad (6)$$

From equations (5) and (6) we obtained the temporal behaviour of the expected values:

$$\frac{d\langle \overline{c} \rangle}{dt} = \langle \overline{\alpha} \rangle \qquad (7)$$

and the temporal behaviour of the covariance matrix:

$$\frac{d\hat{\sigma}}{dt} = \langle \Theta \rangle \hat{\sigma} + \hat{\sigma} \langle \Theta \rangle^T + \langle \hat{\beta} \rangle \qquad (8)$$

where < > symbolizes the expected value, T is the transpose, and Θ is the Jacobian of α:

$$\Theta = \begin{bmatrix} -Ky & -Kx & 0 \\ A & -Bz & -By \\ -C & 0 & 0 \end{bmatrix} \qquad (9)$$

The mesoscopic model is given by the equations (7) and (8). Equation (7) describes the behaviour of expected values of p53, Mdm and D_m concentrations, respectively, and equation (8) describes the internal fluctuations around these.

RESULTS AND DISCUSSION

With the purpose of analyzing the model predictions we selected as control parameters the B constant associated to Mdm2 degradation, and the Φ_2 constant related to the damage level of DNA. In order to simplify, the rest of the constants are assumed equal to 1; thus the equation (7) is written:

$$\begin{aligned} \frac{dx}{dt} &= 1 - xy \\ \frac{dy}{dt} &= x - Byz \\ \frac{dz}{dt} &= \Phi_2 - x \end{aligned} \qquad (10)$$

where the corresponding stationary state is:

$$x_{ss} = \Phi_2, y_{ss} = \frac{1}{\Phi_2}, z_{ss} = \frac{\Phi_2^2}{B} \qquad (11)$$

If the stationary state is substituted in the Jacobian (9), we arrive to the characteristic equation as a function of the eigenvalues λ:

$$B + 2\Phi_2\lambda + \frac{1+\Phi_2^3}{\Phi_2}\lambda^2 + \lambda^3 = 0 \qquad (12)$$

and we find that the periodic oscillations occur because of a supercritical Andronov-Hopf bifurcation [20], where:

$$B_c = 2 + 2\Phi_2^3 \qquad (13)$$

Taken into account that i) the oscillations number of p53 and the probability of apoptosis process depend of level damage, we considered as hypothesis that the apoptosis process is controlled and induced by the sustained oscillations of p53, whereas this process doesn't occur when the oscillations are damped, indicating the survival of the mutated cells.

The bifurcation diagram obtained from equation (13) is shown in Figure 2, where the survival or apoptosis cells are described as a function of control parameters.

In this case, when $B < B_c$, p53 shows damped oscillations (figure 3.a) and the stimulated process is the cells' survival, while when $B > B_c$, the stimulated process is the cells apoptosis, which is just regulated by the p53 sustained oscillations (Figure 3.b). If the hypothesis proposed is correct, the obtained bifurcation diagram can be used to establish different therapy strategies against cancer based on the stimulation of apoptosis.

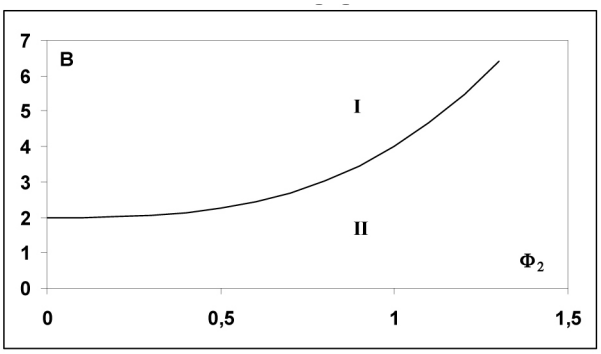


Figure 2. Bifurcation diagram ($B_c = 2 + 2\Phi_2^3$); I undamped oscillation; II damped oscillation.

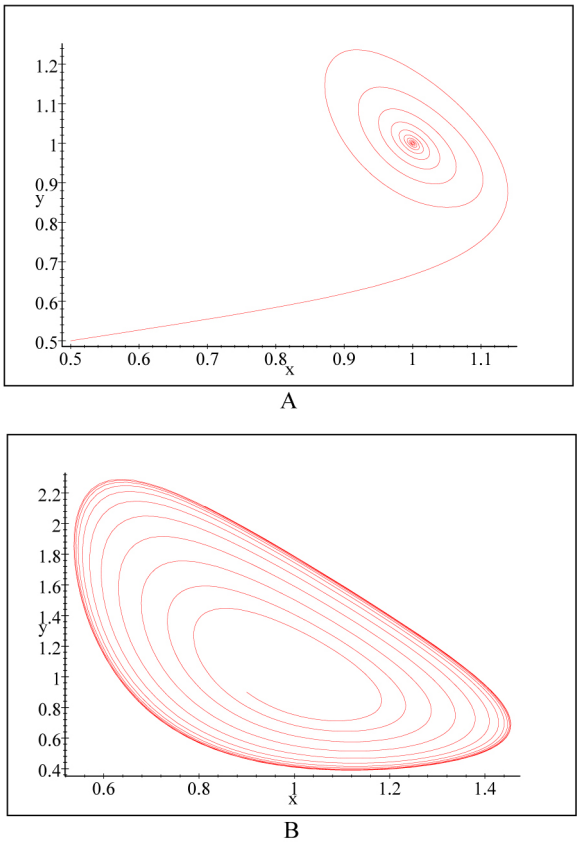


Figure 3. Phase plane x,y corresponding to predicted dynamical behaviour; a) B < B_c, damped oscillation, stable focus (II); b) $B > B_c$, undamped oscillation, limit cycle (I).

The protein Mdm2, which is the biological regulator of p53, is overexpressed in certain types of cancer [21],. Taking into account the bifurcation diagram and the established hypothesis, if the value of the parameter B, which represents the Mdm2 degradation, is increased in such way that $B > B_c$, the apoptosis process is stimulated and regulated through sustained oscillations. This theoretical result may correspond to therapeutic strategies recently established by other authors, which proposed a therapy based on the inhibition of Mdm2 [21,22,23].

The apoptosis process has been associated with high levels of p53, which is based on the experimental results which show p53 increase with damage level [24]. In this sense, the absence of p53 observed in certain cancers, seems to corroborate this

hypothesis. Nevertheless, other types of cancer show high level of p53, which is corre-lated with a poor prognosis [25,26]. According to the proposed model, apoptosis can only occur when $\phi_2 < \phi_{2,c}$ for a given value of B, i.e. the cell survival is induced when the level of p53 is too high, which can explain why a high level of p53 is not always associated to apoptosis.

According to the mesoscopic formalism, the fluctuations magnitude behaviour, which is related to the square root of the covariance matrix determinant, is given by:

$$\det[\sigma] = \frac{\left(\frac{\Phi_2^5 B^4 + 2\Phi_2^6 B^3 + D_1 B^2 + D_2 B + D_3}{\Omega^3 \Phi_2^4 B} \right)^{0.5}}{\left(2 - B + 2\Phi_2^3\right)} \qquad (14)$$

where $\Phi_1 = K = A = C = 1$. In this case, we observed that the bifurcation condition is in the denominator of (14), in such way that:

$$\begin{aligned} B = B_c \quad & (\det\sigma)^{0.5} \to \infty \\ B < B_c \quad & (\det\sigma)^{0.5} > 0 \\ B > B_c \quad & (\det\sigma)^{0.5} < 0 \end{aligned} \qquad (15)$$

The physical meaning is the following: when the sys-tem has a stationary stable state, the dynamic behaviour shows damped oscillations, and the fluctuations magnitude takes a constant and positive value, which scales up with the system size and is increased with B. In the bifurcation point, the variance and covariance take an infinite value, indicating that the fluctuations increase to a macroscopic scale, while for unstable stationary states, the fluctuations magnitude takes a negative value, with no physical meaning.

CONCLUSIONS

We considered as hypothesis that the apoptosis occurs as a result of a non-linear self-organized process far from thermodynamic equilibrium. Based on it, a stochastic formalism that allows a better understanding of the regulation processes of apoptosis through p53 sustained oscillations is proposed, where the obtained deterministic model predicts the reported qualitative experimental results related with the p53 oscillations when there is a DNA damage. It also predicts that it is necessary a strict regulation of p53 level for stimulating the apoptosis process, which depends of both the Mdm2-p53 and the inhibition of Mdm2 negative feed-back loops.

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