An Evolutionary Algorithm for Simulating Genetic Regulatory Response of the P53 Gene

Costin-Radu BOLDEA

Department of Informatics,
West University of Timisoara, Romania
cboldea@info.uvt.ro

Abstract. The paper present a genetic algorithm to simulate p53/mdm2 complex genetic regulatory response, in order to maximize the apoptotic effect of the oscillatory characteristic of this complex on tumor cells. The evolutionary method presented suggest a medical strategy in cancer treatment.
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1 INTRODUCTION

The p53 gene is a tumor-suppressor gene (Vogelstein et al., 2000 [8]) and has been described as "guardian of the genome". It is known to play a vital role in the cell because when it is not functioning correctly results cancer; p53 is dysfunctional in the majority of cancer types, and greater than 18000 different p53 mutations have been found in cancers (Bode and Dong, 2004 [2]). The p53 protein is the major node of a network that works to apply the "brakes" on cell multiplication and in certain cases causes apoptosis. It's primary function is to act as a transcription factor. In a normal unstressed cell the concentration of p53 is kept very low. When stress occurs, the members of the network interact to produce a 3-10 fold increase in the concentration of activated p53. This process is very quick with p53 levels increasing within minutes and the first apoptotic events occurring within a few hours in some cell types.

Because some of the cellular effects of activated p53 can be irreversible, keeping p53 function under tight control in normal cells is critical. A key player in the regulation of p53 is the Mdm2 protein. Inactivation of the mdm2 gene in mice results in early embryonal lethality (Vogelstein et al., 2000 [8]). Conceivably, in the absence of functional Mdmd2 protein, p53 becomes strongly deregulated to the extent that its excess activity leads to embryonic death. On the other hand, excessive Mdm2 expression can lead to constitutive inhibition of p53 and thereby promote cancer without a need to alter the p53 gene itself. Mdm2 exhibits a dual relationship with p53 .

A qualitative study of the time dependence of the concentration of p53 and mdm2 has been carried out in reference [3]. Approximately one hour after

the stress event (i.e., the DNA damage), 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.

This paper use a simple mathematical model of the p53-Mdm2 feedback loop. The purpose of this work is to simulate the gross mechanisms of p53-Mdm2 interactions as presently known, using a computational adaptative investigation. In particular, I show that specific assumptions characterizing the interactions between p53 and Mdm2 lead to an oscillatory behavior of both p53 and Mdm2 protein levels after a sufficiently strong damage signal. In agreement with this simulation, the levels of both proteins are shown to oscillate in irradiated cells. Such oscillation may enable the more effective execution of a reversible p53 response.

2 THE MATHEMATICAL MODEL

The mathematical model used was first proposed by [1] and use a simplified description of the p53-Mdm2 interaction. The negative effect of Mdm2 on p53 protein level and activity includes:

- the inhibition of p53 transcriptional activity and
- the promotion of p53 degradation, mediated through the binding of Mdm2 to the p53 protein.

Activated p53, in turn, up-regulates Mdm2, by enhancing the transcription of the mdm2 gene.

The model of p53/mdm2 complex is sketched in Fig. 1. The total number of p53 molecules, produced at constant rate S, is indicated with p. The amount of the complexes built of p53 bound to mdm2 is called pm. These complexes cause 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.

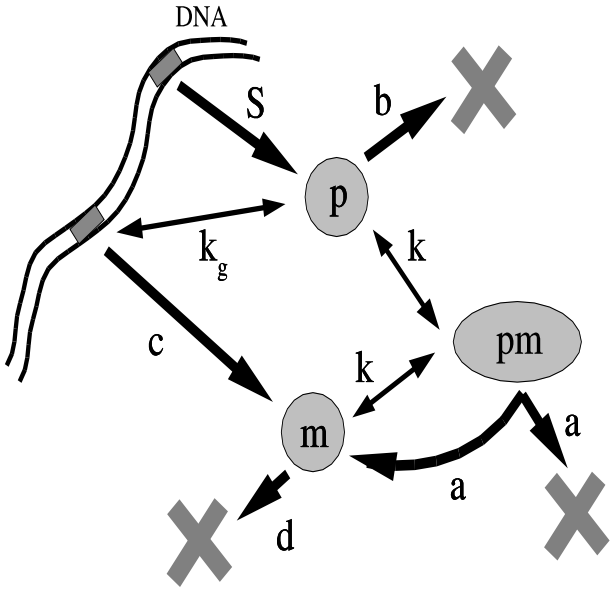

Fig. 1. A sketch of the mechanism which control the amount of p53 in the cell, as described in [1]. The grey crosses indicate that the associated molecule leaves the system.

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 it was allowed 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 ubiquity pathway has been estimated to be $a \approx 3 \cdot 10^{-2} s^{-1}$, while the spontaneous degradation of p53 is $d \approx 10^{-4} s^{-1}$ [2]. The dissociation constant between p53 and mdm2 is $k \approx 180$ (expressed as number of molecules, assuming for the nucleus a volume of $0.6 \mu m^3$), and the dissociation constant between p53 and the mdm2 gene is $k_g \approx 28$ (see [1]). In order to normalize the values for the protein

production rates, it was used typical values, namely $S = 1s^{-1}$ and $c = 1s^{-1}$. 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 stationary condition for equation 1 without delay can be found by the intersection of the curves

$$m(p) = \frac{c(a+b)p - cs}{d(a+b)p - d(S - ak_g} \tag{2}$$

$$m_k(p) = \frac{(S - bp)((a+b)p + ak - S)}{a((a+b)p - S)}, \tag{3}$$

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

$$\frac{dp}{dk} = \frac{dp}{dm}\frac{dm_k}{dk} \approx \frac{d(S - bp)}{ck_g(a+b)}, \tag{4}$$

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

$$\Delta p = \frac{d(S - bp)}{ck_g(a+b)}\Delta k, \tag{5}$$

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

The feedback mechanism that involves the proteins p53 and mdm2, induces cell death as a controlled response to severe DNA damage. The response takes place if the dissociation constant k between p53 and mdm2 varies from its normal value.

The dynamics of the system 1 modify qualitatively in function of the nonzero delay τ. Using as reference the halflife of an RNA molecule, with is of the order of 1200 s (from [3]), we consider $\tau = 1200$. The 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 (implemented in Mathematica 5.0). After the system has reached its stationary state under basal condition, a stress is introduced (at time $t = 20000$ s) by changing instantaneously the dissociation constant k. In Fig. 2 it was displayed 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 50 (c).

The stress model is a pulse of signal that can represent a short exposure of cells to DNA damaging agents: UV or ionizing radiation (IR). The effect of a stress pulse is differentiated in function of the tumor fitness of the cell, increasing the dissociation constant k in the normal case and decreasing it in the tumor case.

When k is increased by any factor, the response is very similar to the response of the system without delay (Fig. 2a), characterizing a normal cell. On the contrary, when k is decreased the system displays an oscillatory behavior, as in the case of a tumor cell. At the value $k \approx 0.1$, for example, the system has a marked response (see also Figs. b and c). The maximum of the first peak takes place approximately 1200s after the stress, which is consistent with the lag–time observed in the experiment [3], and the peaks are separated from ≈ 2300s. A strong variation of the concentration p of p53 gene induce apoptotic cell death as a regulatory response of the p53/mdm2 complex.

We consider the problem of separating the cancerated cell using a stream of short stress radiation impulses u_t, of intensity $q(t)$, in order to amplify the effect described in the b. case from before, kipping alive the rest of the normal cells. But increasing the level of radiation produce severe damages to all the cells, so the problem is reduced to maximize the variation of p, with a low level of radiation. In mathematical terms, we wish to maximize the function

$$f(k) = \frac{\frac{\partial p}{\partial t}}{\sum_t q(t)} \tag{6}$$

using an evolutionary algorithm.

3 EVOLUTIONARY ALGORITHM

Systems based on evolutionary algorithms [4] maintain a population of potential solutions to the problem and apply some selection process based on the quality or *fitness* of individuals, as natural selection does. The population is renewed by replacing individuals with those obtained by applying "genetic" operators to selected individuals. The most usual "genetic" operators are *crossover* and *mutation*. Crossover obtains new individuals by mixing, often in some problem dependent way, two individuals, called parents. Mutation produces a new individual by performing some kind of random change on an individual. The production of new generations continues until resources are exhausted or until some individual in the population is fit enough. Evolutionary algorithms have proved very useful as search and optimization methods.

In our case, individuals represent sequences of stress pulses produced by repeated exposure to different levels of radiation. Then, the individuals of our evolutionary algorithm could be represented as the list

$$\text{Q}= \boxed{q_1}\boxed{q_2}\boxed{\cdots}\boxed{q_n}$$

Each stress pulse produce a perturbation in the stationary solution by the modification of the level of dissociation constant k, as described in [1]:

$$\Delta k = \frac{c_1 q(t)}{1 - c_2 pm} \tag{7}$$

where c_1 is a proportionality constant and c_2 is a small constant.

As the **fitness** function of individual sequences we take a variation of the function f from 6:

$$f(k) = \frac{max(\frac{\partial p}{\partial t})}{\sum_{t=1}^{n} q_i} \tag{8}$$

where the calculus of $max(\frac{\partial p}{\partial t})$ use the numerical method of Adami, implemented in Mathematica 5.0.

The first step of an evolutionary algorithm is the creation of a population of individuals, or potential solutions to the problem. In our case, these individuals represent a set of sequences variable level of stress pulses. The simplest way of creating one such sequence is to random choose the values q_i between 0 and a maximum, normalized here, level 1. The number of the pulses is fixed from the beginning ($n = 20$), and the time between two pulses was taken constant and equal at the halflife of an RNA molecule: 1200 s.

The results of an EA are usually sensitive to settings such as the population size, the number of generations and the rates of application of the crossover and mutation operators.

We have implemented the classic one point crossover, which creates two offspring by combining two individuals in such a way that the first part of one parent up to a crossover point is combined with the second part of the other parent and vice versa. Afterwards, the best offspring substitutes the worst parent. This is a steady state, elitist strategy. The selection of parents pairs are randomized.

The mutation operator is applied to every individual of the population with a probability given by the mutation rate p_{mut}. The mutation operator modify one of the pulse of a stream Q arbitrary between 0 and 1.

4 EXPERIMENTAL RESULTS

Numerical solution of the model equations suggest that, under certain conditions, p53 and Mdm2 undergo damped oscillations after a damage signal (as see in Fig. 2). After 200 "generation" of different stress stream, the winning strategy to maximize the variation of oscillations consist of an initial maximum pulse of stress at $t = 0$, repeated at an interval of the order of $dt = 5$ hours. The result depend strongly on the time delay τ.

It is seen that after the initial pulse, both p53 and Mdm2 levels increase several-fold with respect to their basal levels, to which they return after the damage signal is resolved. A time delay can be seen between the peaking of p53 and Mdm2 levels. (In this particular example, Mdm2 peaks with a delay of one h relative to p53's maximum.) For the solution proposed by the Evolutionary algorithm, maximum peak values p^* and m^* for the amount of p53 and mdm2, respectively, calculated with $\tau = 1200s$, is presented bellow.

k	p^*	m^*
1	47.3	33.6
1.8	49.5	36.8
3	63.9	52.4
15	858.3	96.7
19	287	99.3
180	632	99.6

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, and the maximum effect take place after 20 hours from the first irradiation pulse.

The numerical experiment was repeated 5 times, with similar results.

In conclusion, the method described in this paper confirm numerically 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, and suggest a medical future utilization of the p53/mdm2 complex oscillations.

Acknowledgments

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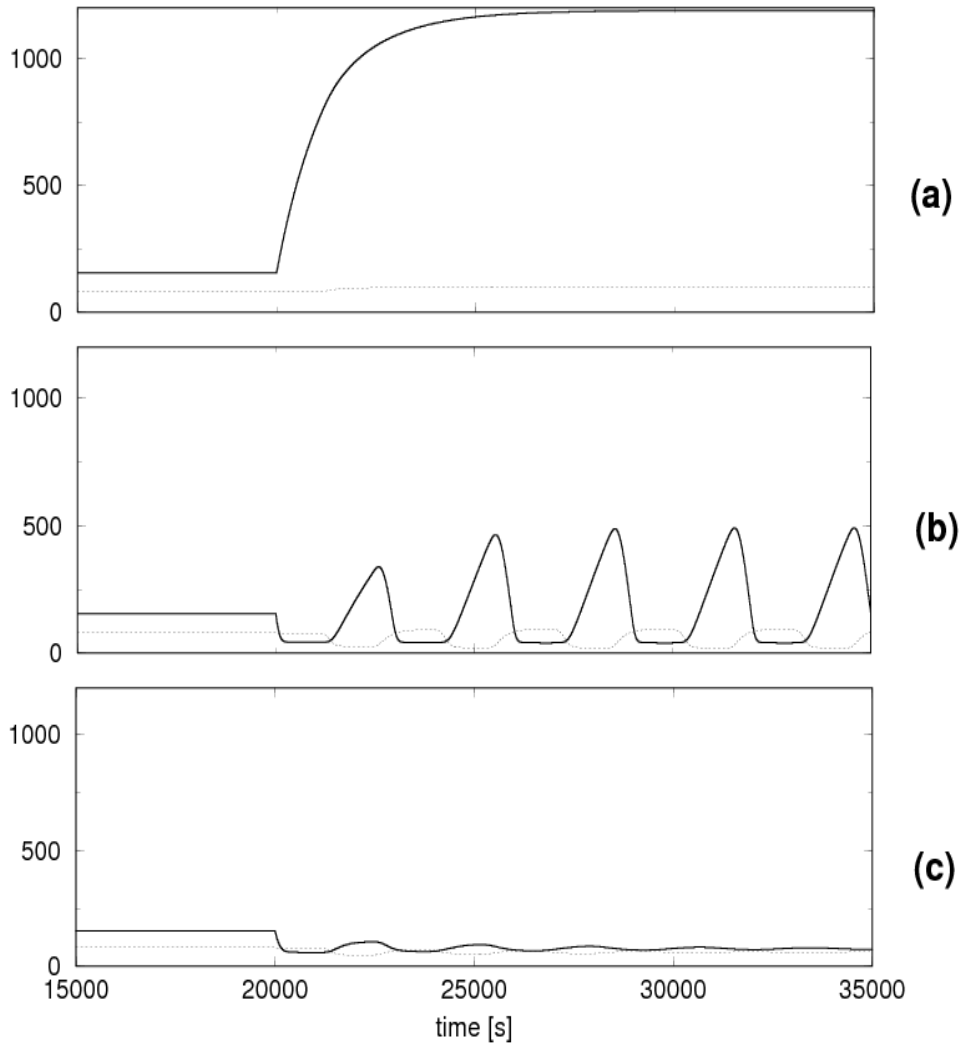

Fig. 2. The response in the concentration of p53 (solid line) and mdm2 (dotted line) upon variation of the dissociation constant k.