A Statistical Study on Oscillatory Protein Expression

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Abstract. Motivated by the experiments on the dynamics of a common network motif, p53 and Mdm2 feedback loop, by Lahav et al. [Nat. Genet **36**, 147(2004)] in individual cells and Lev Bar-or et al. [Proc. Natl. Acad. Sci. USA **97**, 11250(2000)] at the population of cells, we propose a statistical signal-response model with aiming to describe the different oscillatory behaviors for the activities of p53 and Mdm2 proteins both in individual and in population of cells in a unified way. At the cellular level, the activities of p53 and Mdm2 proteins are described by a group of nonlinear dynamical equations where the damage-derived signal is assumed to have the form with abrupt transition ("on" $\leftrightarrow$ "off") as soon as signal strength passes forth and back across a threshold. Each cell responses to the damage with different time duration within which the oscillations persist. For the case of population of cells, the activities of p53 and Mdm2 proteins will be the population average of the individual cells, which results damped oscillations, due to the averaging over the cell population with the different response time.

Keywords: p53-Mdm2 interaction, negative feedback loop, sustained and damped oscillatory dynamics.

1 Introduction

Recently, intensive studies have been devoted to the signal-response relation between DNA damage and gene expressions within living cells. The studies mainly carried out in two directions, one is to extract the general principles of complex protein networks, such as, the interplay between network dynamics and topology; another is to study the dynamics of basic network motifs and to understand their specific functions and structures. Special attentions have been paid to the regulatory oscillatory dynamics of the expressions or activities of the common network motif composed of the tumor suppressor protein p53 and its transcriptional target Mdm2 [1,2,3,4,5].

The experiment at the population of cells [1] has shown that under certain conditions, the activities of the average protein levels of p53 and Mdm2 behave

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damped oscillations in response to DNA damage. The stronger the damage is, the higher and broader the amplitude of the oscillation averagely responds. In contrast, the latest experiment in individual cells [2] sets out to address that in response to ionizing radiation, cells emit p53 in discrete pulses of fixed height and duration that do not depend on the strength of DNA damage (i.e., *sustained* oscillations), and, instead, with different number of pulses for the genetically identical cells. The mean number of pulses increases with the extent of DNA damage.

Several simple theoretical models [1,6,7,8] based on the p53-Mdm2 regulatory negative feedback between the transcription of p53 and Mdm2 proteins have been proposed to qualitatively describe the dynamical behaviors of average protein levels in population of cells. However, we are still far away from understanding the dynamical mechanism for the sustained oscillatory behaviors at individual level, and the relation between the damped oscillatory behaviors in population of cells and undamped oscillatory behaviors in individual cells [2,5]. Moreover, an exponential function in time is generally used to express the signal response to the damage in the case of population of cells [2]. This response relation has far-reaching implication for our understanding of how cells respond to damage in different manners in individual and population cases.

Exploiting regulatory negative feedback loop, in this paper, we propose a statistical model of negative p53-Mdm2 feedback system with the aim to describe such the different dynamical oscillatory behaviors of protein levels both in individual and in population of cells in a self-consistent and unified way. It should be emphasized that different from the previous models [1,6,7,8], the dynamics of damage-derived signal is paid special attention in this paper in addition to taking account of all the knowledge of the biochemical mechanism of the system and to be simplified to the major components in the system, because the dynamics of damage-derived signal might play crucial role in describing the different dynamical activities of the system. At the cellular level, the signal is assumed to have the binary form with abrupt transition ("on" $\leftrightarrow$ "off") as soon as signal strength passes forth and back across a threshold. The time duration when the signal is above the threshold mainly depends on the signal strength, the different manners for cells to response the damage and the repairing abilities of cells, etc. For the case of population of cells, the activities of p53 and Mdm2 proteins will be the ensemble (population) averages of the individual cells, each of which responds damage with different time duration. The average levels of p53 and Mdm2 proteins over cell population will show damped oscillations, due to the averaging of cell population. It will be shown that, under above-mentioned considerations, the experimental results [1,2] of different oscillatory behaviors will be satisfactorily reproduced in this paper.

The paper is organized as follows. In Section 2, a phenomenological dynamical model will be introduced for a negative feedback network of p53-Mdm2 interaction. In Section 3, the numerical results and the analysis will be shown for various conditions. Finally, the last Section will be devoted for discussion and summary.

2 Model

We now present our dynamical model of p53-Mdm2 feedback loop. We assume that the level of p53 protein in a cell obeys the following kinetic equation:

$$\frac{dP(t)}{dt} = S_P - \alpha_P M(t) P(t) \left(1 - \gamma_P \mathcal{S}(t)\right) - \mu_P P(t), \tag{1}$$

On r.h.s. in Eq. (1), the first term describes the synthesis rate of the p53 protein, the second one represents Mdm2- and signal-dependent degradation of p53 and the last one reflects an Mdm2-independent mechanism for p53 degradation. The coefficient α_P represents the ability of Mdm2 to promote p53 degradation, and controls the basal levels of p53. $\mathcal{S}(t)$ is the damage-derived signal which is the key component as described in Section 1. The introduction of parameter γ_P is to take into account of that to what extent the damage-derived signal $\mathcal{S}(t)$ might inhibit the p53 degradation induced by the activation of Mdm2 protein.

$M(t)$ represents the level of Mdm2 protein whose kinetic equation is given as:

$$\frac{dM(t)}{dt} = S_M + \alpha_M \mathcal{T}(t) - \mu_M M(t) \tag{2}$$

Here the coefficient S_M denotes the rate of p53-independent Mdm2 transcription and translation, whereas the last term describes Mdm2 degradation. The coefficient α_M denotes the maximal initiation rate of Mdm2 transcript initiation upregulated by p53 [6]. $\mathcal{T}(t)$ in the second term is a Hill-type function and reads

$$\mathcal{T}(t) = \frac{\{P(t-\tau)\}^N}{K^N + \{P(t-\tau)\}^N}, \tag{3}$$

which takes into account the transcriptional and/or translational time delay, denoting as time τ, between the activation of p53 and the induction of Mdm2. The parameter K corresponds to some sort of threshold-for-activation for p53-protein concentration, and N is a Hill coefficient that determines the steepness of $\mathcal{T}(t)$.

Equations (1) and (2) describe how the nonlinear dynamics of the system depends on the parameters incorporated in the model.

3 Numerical Results and Discussions

The sound experimental results at the cellular level [2] have been considered to determine the parameters, which shows that the width of pulse was 350 ± 160 min(mean $\pm$ s.d.); the timing of the first pulse maximum was rather variable, 360 ± 240 min after damage, but the time between the maxima of two consecutive pulses was more precise, 440 ± 100 min. Mdm2 peaks are with a time delay of ~ 100 min relative to p53 maximum.

However it is still not so easy to define all the model parameters since the experimental data are limited at present. Some parameters should be roughly

estimated phenomenologically, for example, μ_P is taken to be small with respect to the Mdm2-dependent rate of p53 elimination, which reflects the fact that although other mechanisms for the degradation of p53 may exist, a large body of data points to Mdm2 as the key regulator of p53 stability [1]. The first order degradation rate of Mdm2 μ_M could be chosen as 0.05/min, which corresponds to Mdm2 half-lives approximately 20-25min under basal condition.

It has been shown [9] that when the values of the parameters incorporated in the model are changed in a rather large region around the ones used in this paper, the oscillatory solutions of the concentration of p53 and Mdm2 proteins of Eqs. (1) and (2) could be expected and the solutions are rather robust.

3.1 The Case of Individual Cells

When cells are exposed to the damaging agents, such as UV or ionizing radiation, the signal $\mathcal{S}(t)$ will be derived which eventually activates an initial pulse of p53 concentration. From biological point of view, at cellular level, $\mathcal{S}(t)$ can be considered as switch "on" and will be with abrupt transition from "on" to "off" when signal is resolved, as the behavior of the p53-Mdm2 system evolves to give reasonably defined quanta of repair enzymes in response to stress [2]. $\mathcal{S}(t)$ might be defined as a step function in time

$$\mathcal{S}(t) = \Theta(t - \tau_{th}) = \begin{cases} 1 \text{ if } t \leq \tau_{th} \\ 0 \text{ otherwise} \end{cases} \tag{4}$$

where $\tau_{th} = n\tau_{ch}$, and τ_{ch} is the characteristic duration within which the signal stress is in the region of oscillatory response and a pulse is activated. n is a non-negative integer and so that τ_{th} accounts for the total time scale of $\mathcal{S}(t)$. The value of τ_{ch} used in this paper is $\tau_{ch} \approx 410$ minutes which is obtained from the characteristic frequency of the solutions of Eqs. (1) and (2) with the parameters used.

We use the forth-order Runge-Kutta algorithm for integrating the kinetic Eqs. (1) and (2) incorporated with the signal $\mathcal{S}(t)$ in Eq. (4). The initial conditions of $P(t)$ and $M(t)$ are defined by their basal or stationary values in Eq. (6) discussed in the following.

Figure 1 shows the dynamical evolution of the concentration of p53 and Mdm2 proteins for the case in individual cells with $\mathcal{S}(t)$ defined in Eq. (4), which are scaled with their basal values $P(0)$ and $M(0)$. Under normal environment, the amount of p53 protein in the cell is kept low and tightly regulated by a genetic network built of Mdm2 and p53 itself. p53 is produced at an essentially constant rate and promotes the expression of the Mdm2 gene [10]. On the other hand, the Mdm2 protein binds to p53 and promotes its degradation [11], decreasing its concentration. When DNA molecule is damaged, a cascade of events causes phosphorylation of several serines in the p53 protein, which modifies its binding properties to Mdm2 [12]. As a consequence, the cell experiences a sudden increase in the concentration of p53, which activates a group of genes responsible for cell growth arrest and apoptosis. The increase in p53 protein levels and the transcription activity of p53 lead, in turn, to increase the production of Mdm2 with a time delay. Mdm2 protein again promotes the rapid degradation of the

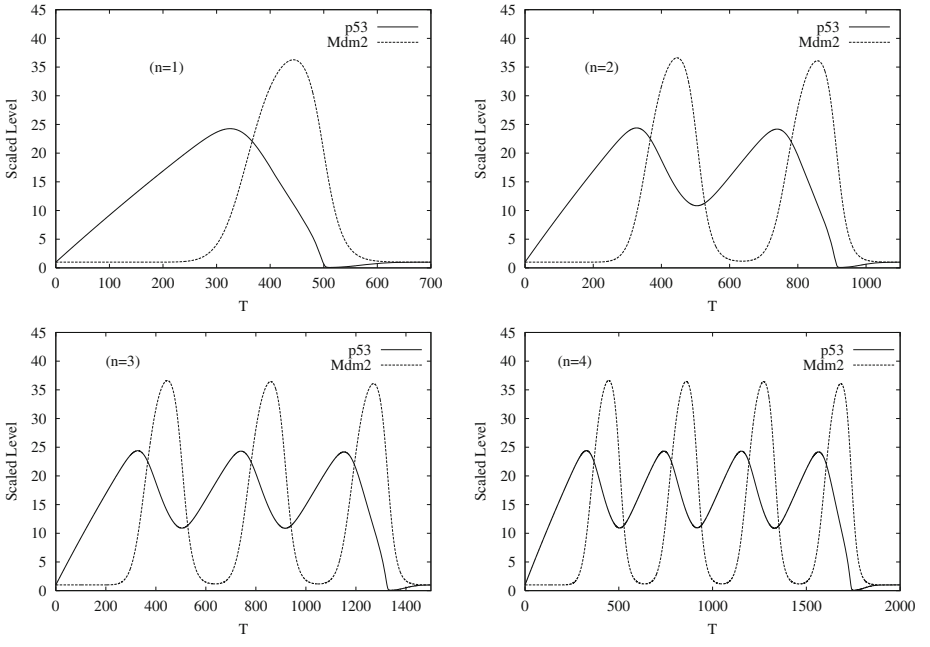

Fig. 1. Concentration of p53 tumor suppressor protein and its transcriptional target Mdm2 relative to their basal levels with the parameters: $S_P = 0.5$, $\alpha_P = 1.8$, $\gamma_P = 0.996$, $\mu_P = 2.5\times10^{-4}$, $S_M = 2.35\times10^{-3}$, $\alpha_M = 0.1$, $\mu_M = 0.05$, $K = 120$, $N = 10$. The initial conditions of $P(t)$ and $M(t)$ are defined by their basal values as $P(0) = 5.89$ and $M(0) = 0.047$. The dynamics of signal $\mathcal{S}(t)$ is described by Eq. (4). The transcriptional time delay τ=100 minutes. Abscissa T denotes a time in units of minute.

p53 protein. Thus the sustained oscillations occur as long as the signal is presented. Here only up to four oscillations are shown in order to compare with the experiment [2]. When the signal is completely resolved, the p53-Mdm2 loop return to normal case and the levels of p53 and Mdm2 to their basal (or stationary) values given in Eq. (6). Thus the finite number of sustained oscillations for individual cells found in experiments [2] can be obtained in a simple way by representing the damage-derived signal in the form, as described by Eq.(4), proposed in this paper.

Noting that the characteristic features of p53 protein levels displayed in Fig. 1 are that the width of each pulse is 328 min; the timing of first pulse maximum at 327 min; the time between first and second pulses 413 min; the time delay $\tau = 100$ min and the peaking of second pulse at 720 min. All those features satisfactorily fit the experimental results reported in [2].

3.2 The Case at the Population of Cells

When we turn to the case at the population of cells, the levels of proteins should be the averages over the Poisson ensemble of individual cells, each member of

which responds to damage with different duration τ_{th} of p53-activating signal $\mathcal{S}(t)$ in Eq. (4) due to the stochastic mechanism in the radiation damage as well as in the gene expression [13,14,15] as mentioned in the Introduction section. When N_e is used to represent the total number of cells in the ensemble, $P^{\tau^i_{th}}(t)$ and $M^{\tau^i_{th}}(t)$ $\{i = 1, 2, \cdots, N_e\}$ are the levels of p53 and Mdm2 proteins for i_{th} cell, which are obtained with the same method as described in the above subsection for the case of individual cells with the time scale τ^i_{th} of the signal $\mathcal{S}(t)$ in Eq. (4). The resultant levels of p53 and Mdm2 proteins averaging over a population of cells can be written as

$$P(t) = \frac{1}{N_e}\sum_{i=1}^{N_e} P^{\tau^i_{th}}(t), \qquad M(t) = \frac{1}{N_e}\sum_{i=1}^{N_e} M^{\tau^i_{th}}(t) \tag{5}$$

where $\tau^i_{th} = n^i \tau_{ch}$. n^i is a non-negative number. In order to consider the stochastic mechanism such as in the radiation damage and in the gene expression, n^i is randomly generated to let τ^i_{th} exponentially distributed according to the Poisson distribution $e^{-\tau^i_{th}/\tau_s}$. It can be easily proved that the parameter τ_s is the average of τ^i_{th} over the Poisson ensemble of individual cells when τ_{ch} is supposed to be a constant since we ignored the difference of various types of damage and the p53-dependent DNA repair processes. Generally speaking, τ_s is expected to be much larger than the characteristic period of oscillation τ_{ch}, as the former characterizes the average time for DNA repair process in the population cells and the latter describes the period of a single oscillation in that process.

Here, it should be mentioned that an exponential form of the signal $\mathcal{S}(t) \sim e^{-t/\tau_s}$ has been used in Ref. [1] for describing the activities of the proteins in cell population. Differently, in this paper, we use the same equations,(1) and (2), to obtain the levels of the proteins for every individual cell (say, $P^{\tau^i_{th}}(t)$ and $M^{\tau^i_{th}}(t)$) where cells respond damage with different time duration τ^i_{th} in Eq. (4). The average levels in population of cells then quite naturally come from the average over all individual cells included in the ensemble as shown in Eq. (5).

From Fig. 2, it is seen that in response to the damage signal, the scaled concentrations of p53 and Mdm2 proteins at the population level undergo damped oscillations with respect to their basal levels, which results from the average over cells with different τ^i_{th}, i.e., the different numbers of pulses. The damage signal is resolved after ≈ 5 days and the levels will then decrease to a certain stationary values (not shown) which represent the basal levels of p53 and Mdm2 proteins under normal conditions. In terms of Eqs. (1)-(3), the stationary values can be estimated with

$$M(t \to \infty) \approx \frac{S_M}{\mu_M}, \qquad P(t \to \infty) \approx \frac{S_P \mu_M}{\alpha_P S_M + \mu_P \mu_M}. \tag{6}$$

With the used parameters, it is easily to obtain $M(t \to \infty) \approx 0.047$ and $P(t \to \infty) \approx 5.89$.

From our numerical calculations, one may clearly see that the damping mechanism on the oscillations results from the average between cells with different

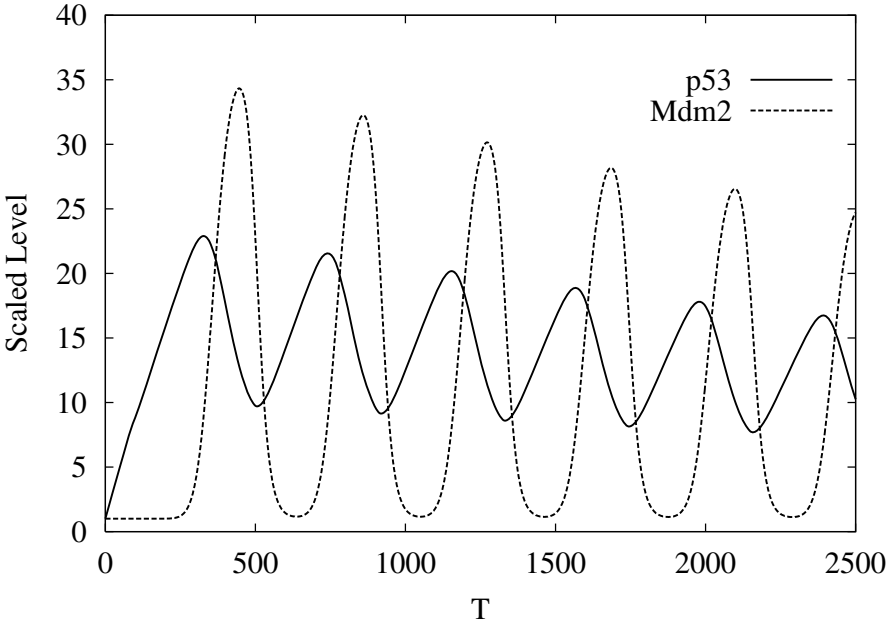

Fig. 2. The levels of p53 and Mdm2 relative to their basal levels obtained with Eq. (5). The parameter τ_s is used as 10 000 minutes. The total number of cells in the ensemble is chosen to be 10 000. Reference of coordinates and the parameter values are the same as in Fig. 1.

τ_{th}^i, i.e., with different numbers of pulses. It is interesting to discuss here about the role of parameter τ_s, whose value might relates with the strength of irradiation (as indicated by the Fig. 3(a) in Ref. [2]) and repairing ability of DNA molecules. If we suppose that the irradiation damage of DNA keeps unresolved (this case might occur in the most severe cases of strong damage), which means that $\mathcal{S}(t)$ keeps constant $\mathcal{S}(0)$ or the parameter $\tau_s \approx \infty$, in such case, the amplitude of oscillations is sustained and the levels show stationary oscillation. When τ_s changes from large through small, cells will be with different τ_{th}^i and the damping mechanism starts to play a role. The smaller τ_s is, the stronger the damping of oscillations.

Origin of oscillation. Here it is worthwhile to clarify the origins of oscillation mechanism. The oscillatory dynamical behaviors could be ascribed as to the regulatory feedback loop in which p53 positively regulates Mdm2 expression while Mdm2 negatively regulates p53 level and activity. And the time delay in p53-dependent induction of Mdm2 should also be crucial for an oscillatory behavior. In order to clarify these points more clearly, let us consider the case without time delay, i.e., $\tau = 0$, which means that the production of Mdm2 is regarded as instantaneously regulated by p53. As shown in Fig. 3, it is clearly seen that levels of p53 and Mdm2 proteins rapidly and simultaneously increase as soon as the signal is turned on and subsequently evolve smoothly and tend to the

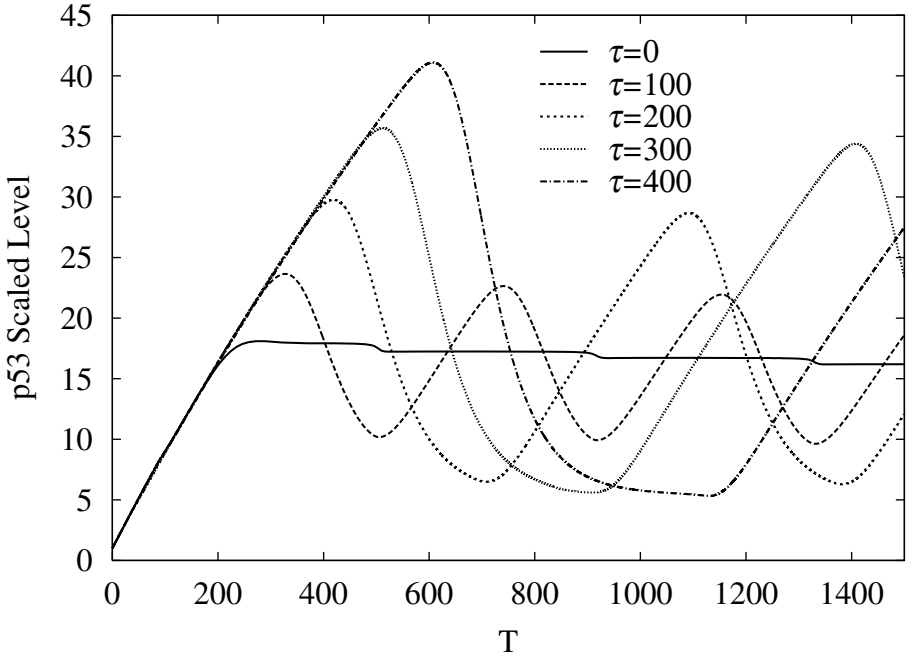

Fig. 3. Effect of time delay in p53-dependent induction of Mdm2 on the protein levels. Reference of coordinates and remaining parameter values are the same as in Fig. 2.

stationary values. There is no oscillation except an initially increasing that could be considered as resulting from the threshold of the parameter K. The effects of time delay also can be seen from Fig. 3 that the patterns of oscillations are quite different for various values of τ. For an intermediate delay ($\tau \approx 1 \sim 3$ hours) as experimental prediction [2], the significant oscillations could be obtained with this model. One might thus conclude that the negative feedback mechanism and time delays could be considered as the main origins in driving the oscillations. Here, it should be mentioned that a similar conclusion has also been reached in Ref. [6,7].

4 Concluding Remarks

We have presented a statistical model of p53-Mdm2 negative feedback loop with a time delay to study the dynamical mechanism of the activities of p53 and Mdm2 proteins in the cases of individual as well as population of cells. It has been shown that both the *sustained* oscillatory dynamics in individual cells and the *damped* one in the population of cells could be explained in a unified way when the dynamics of damage-derived signal is properly introduced. We would like to emphasize that in our present work we try to keep our model with as few free parameters as possible, as the available experimental information is rather

limited so far. However, it can easily be extended to consider more complex network when more experimental data are to come.

It has been clarified that the origin of oscillation mechanism could be ascribed as the nonlinear dynamics of the regulatory negative feedback loop and the time delay in p53-dependent induction of Mdm2. Meanwhile, the damping on oscillation might be considered as resulting from the averaging between cells with different response time to the damage.

This study may provide us with a general understanding of the oscillatory dynamics found in various physical, chemical and biological systems. From this study we can gain certain information on the mechanism of the cellular response to the radiation damage which may have the significance in gene therapy and some other applications.

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