The Dynamics of Stress p53-Mdm2 Network Regulated by p300 and HDAC1

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Abstract

We construct a stress p53-Mdm2-p300-HDAC1 regulatory network that is activated and stabilised by two regulatory proteins, p300 and HDAC1. Different activation levels of p53 observed due to these regulators during stress condition have been investigated using a deterministic as well as a stochastic approach to understand how the cell responds during stress conditions. We found that these regulators help in adjusting p53 to different conditions as identified by various oscillatory states, namely fixed point oscillations, damped oscillations and sustain oscillations. On assessing the impact of p300 on p53-Mdm2 network we identified three states: first stabilised or normal condition where the impact of p300 is negligible, second an interim region where p53 is activated due to interaction between p53 and p300, and finally the third regime where excess of p300 leads to cell stress condition. Similarly evaluation of HDAC1 on our model led to identification of the above three distinct states. Also we observe that noise in stochastic cellular system helps to reach each oscillatory state quicker than those in deterministic case. The constructed model validated different experimental findings qualitatively.

Introduction

The p53 is a 20-Kb tumor suppressor gene located on the small arm of human chromosome 17 that acts as a hub for a network of signalling pathways essential for cell growth regulation and apoptosis. It comprises of 393 amino acids and is divided into several structural and functional domains: the transactivation domain (TAD; residues 1–40), the proline-rich domain (PRD; residues 61–94), the DNA-binding domain (DBD; residues 100–300), the tetramerization domain (4D; residues 324–355) and the C-terminal regulatory domain (CTD; residues 360–393) [1]. Over the recent years many names have been accredited to p53 viz. Guardian of the Genome [2]; Death Star [3] and Cellular Gatekeeper [4] and is regulated by a number of cellular proteins [5]. It is well established that p53 is accountable for preventing improper cell proliferation and maintaining genome integration following genotoxic stress. In normal proliferating cells, p53 is kept in low concentrations and exists mainly in an inactive latent form with a short half-life of 15–30 minutes [6]. This is due to interaction between p53 and Mdm2 the predominant negative regulator of p53. However, cellular insults activates p53 and its level increases rapidly. The activation of p53 is a result of several posttranslational modifications including phosphorylation, acetylation, sumoylation and neddylation [7]. Phosphorylation of Ser-15 and 37 at the amino terminus of p53 prevents Mdm2 binding, thus stabilizing p53. Also phosphorylation at Ser-15 increases p53 affinity for p300, thus promoting acetylation of p53 carboxy terminal by p300 [8]. Further the p53 in-turn activates the p53-targeted genes including those involved in cell cycle arrest and DNA repair, as well as apoptosis and senescence related genes. The activation of the p53-targeted genes leads to cell cycle arrest that forces cell to choose either to repair the DNA damage to restore its normal function or cell death (apoptosis). Further, it has been observed that p53 acetylation is a reversible process and for it Mdm2 recruits HDAC1 (a histone deacetylase) to form a Mdm2-HDAC1 complex which deacetylates p53. Interestingly, it was also shown that p300 can form a complex with Mdm2 in vitro and in vivo [9,10] and this complex (Mdm2-p300) facilitate Mdm2 mediated p53 degradation. Moreover, it has also been reported that Mdm2-p53-p300 complex exists that is also thought to promote ubiquitylation and degradation of p53 [11]. Thus p300 plays dual role and exerts two opposite effects on p53 in cells i.e., it can either interact with Mdm2 promoting Mdm2-mediated ubiquitylation and degradation of p53 [9] or acetylate and stabilize p53. This remains puzzling.

There have been different mathematical techniques to study cellular and sub-cellular processes such as deterministic and stochastic models [12,13]. Stochastic model provide detail picture of molecular interaction in the microscopic systems (small systems with small number of molecules accomodated in each system) that leads the system dynamics as noise-driven process [13,14]. The model further highlights the important role of noise in the system
dynamics, for example detection and amplification of weak noise, the phenomenon known as stochastic resonance [15,16], lifting of cellular expression at different distinct expression state [17] and noise in gene expression can drive stochastic switching among such states [10,19], noise induced stochastic phenotypic switching to different new level in living cells [20] etc. However, deterministic model provides qualitative picture of the cellular or sub-cellular processes.

The aim of the present study is (i) to understand some of the basic issues of p53 autoregulation induced by regulators p300 and HDAC1, (ii) to elucidate the functional relationship of p300 and HDAC1 in regulating p53 function, (iii) how do these regulators lifts the normal p53-Mdm2 network to different stress states and (iv) what could be the role of noise in such situations.

Materials and Methods

Stress p53—Mdm2 model regulated by p300 and HDAC1

In normal proliferating cells, p53 is usually maintained at low levels due to p53 and Mdm2 protein feedback mechanism [21]. In unstressed condition the p53 binds to the regulatory region of Mdm2 gene and stimulates its transcription into messenger RNA (mRNA) with a transcription rate constant \( k_1 \), followed by translation into Mdm2 protein with a rate constant \( k_2 \) [22]. The degradation of Mdm2-mRNA, Mdm2 and genesis of p53 occurs with basal rate of \( k_4 \), \( k_5 \) and \( k_6 \) respectively. The Mdm2 protein then interacts physically with p53 to form Mdm2-p53 complex with the rate of \( k_8 \). Mdm2 functions as an E3 ubiquitin ligase and brings about ubiquitylation of multiple lysine residues (K370, K372, K373, K381, K382 and K386) [23] present in the C-terminal domain of p53 [11]. The ubiquitylation marks p53 for degradation via the 26S proteasome, with rate \( k_7 \). The Mdm2-p53 complex can also dissociate to Mdm2 and p53 with rate constant \( k_9 \). Mdm2 and p300 have been shown to interact with rate constant \( k_{10} \) to form Mdm2-p300 complex, which facilitates p53 polyubiquitination and degradation at rate constant of \( k_1 \) [9,24]. Although there is no direct evidence reported to the best of author’s knowledge in the literature for the degradation of Mdm2-p300 complex, however it has been shown that the p19ARF-binding domain of Mdm2 overlaps with its p300-binding domain suggesting that p19ARF could interfere with the Mdm2/p300 interaction [9]. Therefore, we can assume it is possible that Mdm2-p300 complex can be broken so as to interact with other proteins. Thus in normal unstressed cell, p53 is maintained at low level in an active state with short half-life of 15–30 minutes by Mdm2 and the cells are able to proliferate.

However, under stressed conditions the p53 is stabilized through various post translational modifications which lead to increase its level. Of the various mechanisms, phosphorylation of p53 is the most well studied and it is reported that multiple kinases phosphorylate various residues which increase the level of p53 protein. One of these protein kinases is ATM which upon activation by DNA damage phosphorylates p53 with a rate \( k_{12} \) at serine 15 [25] which is critical for p53 activation and stabilization. Strikingly, the phosphorylation of serine 15 mediated by ATM acts as a nucleation event that promotes subsequent sequential modification of many residues. To achieve this, interconversion of inactivated and activated ATM takes place, with rate constants \( k_{10} \) and \( k_{11} \) respectively. The ATM-initiated phosphorylation reduces the affinity of p53 for Mdm2 while increases interactions with HATs like CBP/p300 [8,26]. Consequently, dephosphorylation of p53 with a rate \( k_{13} \) also takes place to counter this phosphorylation. It has been demonstrated that p300 protein is a co-activator of p53 which potentiates its transcriptional activity as well as biological function in vivo [27]. However, it has also been shown that formation of p300-Mdm2-p53 ternary complex leads to suppressing p53 acetylation and activation [28]. The transcription activation domain (TAD) of p53 binds tightly to p300 with formation rate constant \( k_{15} \). The p53-p300 complex hence formed, causes p53 acetylation with rate constant \( k_{16} \) at multiple lysine residues (K370, K372, K373, K381, K382) of its C-terminal regulatory domain [27,29]. The lysine residues (K370, K372, K373, K381, and K382) are the common sites for both acetylation and ubiquitination [30,31]. Thus their acetylation causes the inhibition of ubiquitination resulting p53 protein stability which is evident from the observation that acetylated p53 has half-life of greater than two hours [32]. Simultaneously, formation and degradation of p300 occurs with rate constants \( k_{13} \) and \( k_{14} \) respectively. Mdm2, p53 and p300 have also been demonstrated to exist in a ternary complex (k18) which is incapable of acetylating p53 [28]. In the complex, TAD1 domain of p53 interacts with Mdm2 while TAD2 interacts with p300 [11]. As mentioned earlier, phosphorylation increases the affinity of p53 towards p300 while decreasing its affinity towards Mdm2. After phosphorylation, the ternary complex dissociates, with rate constant \( k_{21} \) into Mdm2 and p53—p300 complex, in which both TAD1 and TAD2 of p53 interact with p300 [11]. p300 can then acetylate and stabilize p53. Stabilized p53 functions as a tumor suppressor and induces high levels of Mdm2, which in turn promotes p53 degradation by recruiting a p53 deacetylase, HDAC1 with rate constant \( k_{24} \). HDAC1 binds Mdm2 in a p53 dependent manner with binding rate constant \( k_{18} \) and deacetylates p53 with rate constant \( k_{17} \) at all known acetylated lysines in vivo [33]. Moreover, analysis has indicated the presence of MDM2, SMAR1 and HDAC1 complex under conditions of inhibited translation only 12 h post damage rescue while there is lack of complex formation 24 h post damage rescue, thereby suggesting degradation of the Mdm2-HDAC1 complex [34]. HDAC1 is generated and degraded in cells with rate constants \( k_{24} \) and \( k_{22} \) respectively. The unmodified lysine residues can then serve as the substrates for Mdm2-mediated ubiquitylation resulting in p53 degradation and thus completing the feedback loop. The molecular species involved in the biochemical network are listed in Table 1 and the chemical reaction channels in the network are shown in Table 2. The schematic picture of the stress p53—Mdm2 autoregulatory biochemical reaction network model via p300 and HDAC1 based on the experimental evidences and reports mentioned above is shown in Fig. 1.

Stochastic description of biochemical reaction network

We now consider a configurational state \( \bar{X}(t) = (X_1,X_2,\ldots,X_V)^T \) of the system of size \( V \) at any instant of time \( t \) defined by \( N \) molecular species undergoing \( M \) elementary reactions. The change in configurational state during any interval of time \( [t,t+\Delta t] \) is due to random interaction of the participating molecules that leads to decay and creation of specific molecular species in state vector \( \bar{X}(t) \) during the time interval \([13,14,35]\). Therefore the trajectory of this state vector \( \bar{X}(t) \) as a function of time in the configurational space follows Markov process [13,14] and the dynamics of this vector becomes noise-induced stochastic process [13]. If we define \( P(\bar{X},t) \) as the configurational probability of obtaining the state \( \bar{X} \) at time \( t \), then the time evolution of \( P(\bar{X},t) \) will obey Master equation [13,14,36]. Even though the Master equation for complex system could be very difficult to solve analytically, different algorithms have been devised to solve the system dynamics numerically depending on
the nature of the system. For example, stochastic simulation algorithm (Gillespie algorithm) for reaction system without considering time delay [33], stochastic simulation algorithm for time delay reaction system [37,38], τ-leap algorithm which is approximated algorithm of stochastic simulation algorithm for very complex reaction network [39], hybrid algorithm for reaction networks consisting of both slow and fast reactions [40] etc.

The Master equation for the stochastic system can be approximated to simpler Chemical Langevin equations (CLE) based on two important realistic approximations applied on the the system [41]. This can be done by defining a function \( F(\mathbf{X}, \Delta t) \) which is the number of a particular reaction fired during the time interval \([t, t+\Delta t]\) with \( \Delta t \) when applying the two approximations: first approximating \( \lim_{\Delta t \to 0} F(\mathbf{X}, \Delta t) \) which leads to the propensity functions \( \omega \) of the reactions fired remain constant during the time interval, and secondly approximating \( \lim_{\Delta t \to 0} \mathbf{X} \mathbb{N} F(\mathbf{X}, \Delta t) \) condition which gives rise \( \mathbb{V} = 1 \) [41]. These two conditions are true for large population size of each variables in state vector \( \mathbf{X} \) which is valid for natural systems. These two conditions allow the function \( F \) to approximate to Poisson distribution function and then to Normal distribution function with same mean and standard deviation. If the molecular concentration is defined by \( \mathbb{E}(\mathbf{X}) = \frac{1}{V} \mathbb{V} \mathbf{X} \) and linearize Normal distribution function, the Master equation leads to the following CLE of the vector \( \mathbb{E}(\mathbf{X}) \),

\[
\begin{align*}
\frac{d\mathbb{E}(\mathbf{X})}{dt} = & G[\omega(\mathbb{E}(\mathbf{X}), v)] + H[\omega(\mathbb{E}(\mathbf{X}), v), \xi, V] \\
\end{align*}
\]

where, \( G = \sum_{i=1}^{M} v_i \omega_i [x(t)] \) is the macroscopic contribution term and \( H = \frac{1}{\sqrt{V}} \sum_{i=1}^{M} v_i [\omega_i \{ x(t) \}]^{1/2} \) is the stochastic contribution term to the dynamics. \( \xi_i = \lim_{\Delta t \to 0} N_i(0,1)/\sqrt{\Delta t} \) is uncorrelated, statistically independent random noise parameters which satisfy \( \xi(t) \xi(t') = \delta_{tt'} \). \( \{ v \} \) is the stoichiometric matrix of the reactions in the network.

Table 1. List of molecular species.

| S.No. | Species Name | Description | Notation |
|-------|--------------|-------------|----------|
| 1.    | p53          | Unbounded p53 protein | \( x_1 \) |
| 2.    | Mdm2         | Unbounded Mdm2 protein | \( x_2 \) |
| 3.    | Mdm2_mRNA    | Mdm2 messenger mRNA | \( x_3 \) |
| 4.    | Mdm2_p53     | Mdm2 with p53 complex | \( x_4 \) |
| 5.    | ATM_F        | Inactivated ATM protein | \( x_5 \) |
| 6.    | ATM_A        | Activated ATM protein | \( x_6 \) |
| 7.    | p53_P        | Phosphorylated p53 protein | \( x_7 \) |
| 8.    | p300         | Unbounded p300 protein | \( x_8 \) |
| 9.    | p53\_p300\_P| Phosphorylated p53\_p300 complex | \( x_9 \) |
| 10.   | p53\_A       | Acetylated p53 (capped p53) | \( x_{10} \) |
| 11.   | HDAC1        | Unbounded HDAC1 protein | \( x_{11} \) |
| 12.   | Mdm2\_HDAC1  | Mdm2 and HDAC1 complex | \( x_{12} \) |
| 13.   | Mdm2\_p53\_p300 | Mdm2, p53 and p300 complex | \( x_{13} \) |
| 14.   | Mdm2\_p300   | Mdm2 and p300 complex | \( x_{14} \) |

The classical deterministic equations can be obtained from the CLE equation (1) at thermodynamics limit [41] i.e. at \( V \to \infty \), \( N \to \infty \) but \( N/V = \text{constant} \). This leads to \( H \to 0 \) and the equation (1) becomes noise free deterministic equation,

\[
\frac{d\mathbb{E}(\mathbf{X})}{dt} = G[\omega(\mathbb{E}(\mathbf{X}), v)]
\]

(2)

The same equation (2) can also be retrieved from the biochemical reaction network by translating them into a set of differential equations based on standard principles of Mass-action law of biochemical reaction kinetics.

The stress \( p53-Mdm2-p300-HDAC1 \) model network we study is defined by \( N = 14 \) (14 molecular species) and \( M = 24 \) (24 reaction channels). The molecular species, possible reactions, kinetic laws and the rate constants in this model are listed in Table 1 and Table 2 respectively. The state vector at any instant of time \( t \) is given by, \( \mathbb{E}(\mathbf{X}) = (x_1, \ldots, x_{14})^T \), where the variables in the vector are various proteins and their complexes which are listed in Table 1. The classical deterministic equations constructed from these reaction network are given by,

\[
\begin{align*}
\frac{dx_1}{dt} & = -k_1 x_1 x_{14} + k_6 - k_9 x_1 x_2 + k_9 x_4 \\
& - k_{12} x_1 x_6 + k_{13} x_7 + k_{17} x_{10} x_{12} \\
\frac{dx_2}{dt} & = k_2 x_3 - k_5 x_2 + k_7 x_4 - k_8 x_1 x_2 \\
& + k_9 x_4 - k_{18} x_2 x_{11} - k_{20} x_2 x_8 + k_{21} x_{13} \\
\frac{dx_3}{dt} & = k_3 x_1 x_4 \\
\frac{dx_4}{dt} & = -k_7 x_4 + k_8 x_1 x_2 - k_9 x_4 - k_{10} x_4 x_8 \\
\frac{dx_5}{dt} & = -k_{10} x_5 + k_{11} x_6 \\
\frac{dx_6}{dt} & = k_{10} x_5 - k_{11} x_6 - k_{12} x_1 x_6 \\
\frac{dx_7}{dt} & = k_{12} x_1 x_6 - k_{13} x_7 - k_{15} x_7 x_8 \\
\frac{dx_8}{dt} & = -k_{14} x_8 - k_{15} x_8 x_7 - k_{19} x_4 x_8 - k_{20} x_2 x_8 + k_{23} \\
\frac{dx_9}{dt} & = k_{15} x_8 x_7 - k_{16} x_9 + k_{21} x_{13} \\
\end{align*}
\]

(3)
Table 2. List of chemical reaction, propensity function and their rate constant.

| S.No | Reaction | Name of the process | Kinetic law | Rate constant | References |
|------|----------|---------------------|-------------|---------------|------------|
| 1    | \( x_1 + x_{14} \rightarrow \phi \) | p53 degradation | \( k_1 (x_1) (x_{14}) \) | \( 8.25 \times 10^{-4} \text{sec}^{-1} \) | [9,24]. |
| 2    | \( x_2 \rightarrow x_3 + x_2 \) | Mdm2 creation | \( k_2 (x_3) \) | \( 4.95 \times 10^{-4} \text{sec}^{-1} \) | [22]. |
| 3    | \( x_1 \rightarrow x_2 + x_2 \) | Mdm2:mRNA creation | \( k_3 (x_1) \) | \( 1.0 \times 10^{-4} \text{sec}^{-1} \) | [22]. |
| 4    | \( x_2 \rightarrow \phi \) | Mdm2 degradation | \( k_4 (x_2) \) | \( 1.0 \times 10^{-4} \text{sec}^{-1} \) | [22]. |
| 5    | \( x_2 \rightarrow \phi \) | p53 synthesis | \( k_5 \) | \( 0.078 \text{sec}^{-1} \) | [22]. |
| 6    | \( x_2 \rightarrow x_8 \) | Mdm2:p53 degradation | \( k_5 (x_2) \) | \( 8.25 \times 10^{-4} \text{sec}^{-1} \) | [11,23]. |
| 7    | \( x_1 + x_2 \rightarrow x_4 \) | Mdm2:p53 synthesis | \( k_3 (x_1) (x_2) \) | \( 11.55 \times 10^{-4} \text{sec}^{-1} \) | [22]. |
| 8    | \( x_4 \rightarrow x_1 + x_1 \) | Mdm2:p53 dissociation | \( k_4 (x_4) \) | \( 11.55 \times 10^{-4} \text{sec}^{-1} \) | [22]. |
| 9    | \( x_5 \rightarrow x_6 \) | ATM activation | \( k_6 (x_5) \) | \( 1.0 \times 10^{-4} \text{sec}^{-1} \) | [12,23]. |
| 10   | \( x_8 \rightarrow x_9 \) | ATM deactivation | \( k_7 (x_8) \) | \( 5.0 \times 10^{-4} \text{sec}^{-1} \) | [12,23]. |
| 11   | \( x_1 + x_4 \rightarrow x_6 + x_6 \) | Phosphorylation of p53 | \( k_8 (x_1) (x_4) \) | \( 5.0 \times 10^{-4} \text{sec}^{-1} \) | [23]. |
| 12   | \( x_7 \rightarrow x_1 + x_3 \) | Dephosphorylation of p53 | \( k_9 (x_7) \) | \( 5.0 \times 10^{-4} \text{sec}^{-1} \) | [12,23]. |
| 13   | \( x_8 \rightarrow x_9 \) | p300 degradation | \( k_{10} (x_8) \) | \( 1.0 \times 10^{-4} \text{sec}^{-1} \) | [30,31]. |
| 14   | \( x_7 \rightarrow x_9 \) | p53-p300 formation | \( k_{11} (x_7) (x_9) \) | \( 1.0 \times 10^{-4} \text{sec}^{-1} \) | [28]. |
| 15   | \( x_8 \rightarrow x_9 \) | Acetylation of p53 | \( k_{12} (x_8) \) | \( 1.0 \times 10^{-4} \text{sec}^{-1} \) | [27,29]. |
| 16   | \( x_{10} \rightarrow x_1 \) | Deacetylation of p53 | \( k_{13} (x_{10}) (x_1) \) | \( 1.0 \times 10^{-4} \text{sec}^{-1} \) | [29]. |
| 17   | \( x_{11} \rightarrow x_{12} \) | Creation of Mdm2-HDAC1 | \( k_{14} (x_{12}) \) | \( 2.0 \times 10^{-4} \text{sec}^{-1} \) | [29]. |
| 18   | \( x_4 \rightarrow x_8 \) | Creation of Mdm2-mRNA | \( k_{15} (x_4) (x_8) \) | \( 5.0 \times 10^{-4} \text{sec}^{-1} \) | [28]. |
| 19   | \( x_{12} \rightarrow x_{13} \) | Formation of Mdm2-p53-p300 | \( k_{16} (x_{13}) \) | \( 5.0 \times 10^{-4} \text{sec}^{-1} \) | [9,22]. |
| 20   | \( x_3 \rightarrow x_8 \) | Dissociation of Mdm2-p53-p300 | \( k_{17} (x_3) \) | \( 1.0 \times 10^{-4} \text{sec}^{-1} \) | [11,28]. |
| 21   | \( x_{11} \rightarrow x_{12} \) | Degradation of HDAC1 | \( k_{18} (x_{12}) \) | \( 1.0 \times 10^{-4} \text{sec}^{-1} \) | [29]. |
| 22   | \( \phi \rightarrow x_{14} \) | p300 synthesis | \( k_{19} (x_{14}) \) | \( 0.08 \text{sec}^{-1} \) | [30,31]. |
| 23   | \( \phi \rightarrow x_{13} \) | HDAC1 synthesis | \( k_{20} (k_{HDAC1}) \) | \( 2.0 \times 10^{-4} \text{sec}^{-1} \) | [29]. |

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where, \( \{k_i\} \) and \( \{x_i\} \), \( i = 1,2,\ldots,N=14 \) represent the sets of rate constants of the reactions listed in Table 2 and concentrations of the molecular populations listed in Table 1.

Following the same procedure as we have discussed above, we reach the following CLE for the network shown in Fig. 1, Table 1 and Table 2.

\[
\frac{dx_{10}}{dt} = k_{16} x_9 - k_{17} x_{10} x_{12}
\]

\[
\frac{dx_{11}}{dt} = -k_{18} x_2 x_{11} - k_{22} x_{11} + k_{24}
\]

\[
\frac{dx_{12}}{dt} = -k_{17} x_{10} x_{12} + k_{18} x_2 x_{11}
\]

\[
\frac{dx_{13}}{dt} = k_{19} x_4 x_8 - k_{21} x_{13}
\]

\[
\frac{dx_{14}}{dt} = -k_{1} x_1 x_{14} + k_{20} x_2 x_8
\]
Figure 1. Biochemical network model of stress p53-Mdm2-p300-HDAC1. The schematic diagram of stress p53-Mdm2-p300-HDAC1 model. doi:10.1371/journal.pone.0052736.g001

\[ \frac{dx_2}{dt} = k_2x_3 - k_5x_2 + k_7x_4 - k_6x_1x_2 + k_9x_4 \\
- k_{18}x_2x_{11} - k_{20}x_2x_8 + k_{21}x_{13} + \frac{1}{\sqrt{V}} \left[ \sqrt{k_{2}x_{3}x_{8} - \sqrt{k_{5}x_{2}x_{9} + \sqrt{k_{7}x_{4}x_{10} - \sqrt{k_{6}x_{1}x_{2}x_{11}} + \sqrt{k_{9}x_{4}x_{12}}} \right] \\
+ \frac{1}{\sqrt{V}} \left[ - \sqrt{k_{18}x_{2}x_{11}x_{13}} - \sqrt{k_{20}x_{2}x_{8}x_{14} - \sqrt{k_{21}x_{13}x_{15}}} \right] \]  

(18)

\[ \frac{dx_3}{dt} = k_3x_1 - k_4x_3 + \frac{1}{\sqrt{V}} \left[ \sqrt{k_3x_1x_{16} - \sqrt{k_4x_3x_{17}}} \right] \]  

(19)

\[ \frac{dx_4}{dt} = -k_7x_4 + k_8x_1x_2 - k_9x_4k_10x_4x_9 + \frac{1}{\sqrt{V}} \left[ - \sqrt{k_3x_4x_{18}} + \sqrt{k_8x_1x_2x_{19}} - \sqrt{k_{9}x_{4}x_{20} - \sqrt{k_{10}x_{4}x_{8}x_{21}}} \right] \]  

(20)

\[ \frac{dx_5}{dt} = -k_{10}x_5 + k_{11}x_6 + \frac{1}{\sqrt{V}} \left[ - \sqrt{k_{10}x_5x_{22}} + \sqrt{k_{11}x_6x_{23}} \right] \]  

(21)

\[ \frac{dx_6}{dt} = k_{10}x_5 - k_{11}x_6 - k_{12}x_1x_6 \\
+ \frac{1}{\sqrt{V}} \left[ \sqrt{k_{10}x_5x_{24} - \sqrt{k_{11}x_6x_{25} - \sqrt{k_{12}x_1x_6x_{26}}}} \right] \]  

(22)

\[ \frac{dx_7}{dt} = k_{12}x_1x_6 - k_{13}x_7 - k_{15}x_7x_8 \\
+ \frac{1}{\sqrt{V}} \left[ \sqrt{k_{12}x_1x_6x_{27} - \sqrt{k_{13}x_7x_{28} - \sqrt{k_{15}x_7x_8x_{29}}}} \right] \]  

(23)

\[ \frac{dx_8}{dt} = -k_{14}x_8 - k_{15}x_8x_7 - k_{19}x_4x_8 - k_{20}x_2x_8 + k_{23} + \frac{1}{\sqrt{V}} \left[ - \sqrt{k_{14}x_8x_{30} - \sqrt{k_{15}x_8x_7x_{31} - \sqrt{k_{19}x_4x_8x_{32} - \sqrt{k_{20}x_2x_8x_{33} - \sqrt{k_{23}x_{34}}}}} \right] \]  

(24)

\[ \frac{dx_9}{dt} = k_{15}x_8x_7 - k_{16}x_9 + k_{21}x_{13} \\
+ \frac{1}{\sqrt{V}} \left[ \sqrt{k_{15}x_8x_7x_{35} - \sqrt{k_{16}x_9x_{36} - \sqrt{k_{21}x_{13}x_{37}}}} \right] \]  

(25)
indicated by its constant level (\(\ast\) of stabilization. However, when for some time (\(\sim 30\) hours) and then resumes its normal condition indicated by its constant level (\(\sim 12.4\)) which is the level of stabilization. The range of activation is increased as \(kp_{300}\) increases (increase of \(p300\) concentration) as well as there is rise in the level of stabilization. However, when \(kp_{300} \approx 0.06 - 0.08\), p53 maintains sustain oscillations which leads to increasing level of activation as a consequence. With further increment of \(p300\) concentration level, p53 dynamics that was at sustain oscillations switched to damped oscillations and subsequently p53 concentration is stabilized at a constant level. This activity suggests that the capping of the c-terminal of p53 is higher and there is no decrement in the p53 levels as a result of which p53 is stabilized. The results obtained are consistent with the experimental observations which indicates that acetylation of p53 is responsible for its activation [27,31] and stabilization [29,32]. If we further increase the value of \(kp_{300}\), p53 activation decreases maintaining p53 stability but at higher values (\(\geq 80\)). Hence we identify two conditions where p53 is stabilized, one at lower values (nearly normal cell condition) and the other at larger values (cell death condition) of \(kp_{300}\) and in between p53 is activated.

Similarly, Mdm2 dynamics as a function of time for different values of \(p300\) concentration levels is shown (Fig. 3) that demonstrates counter behaviour as expected. The two dimensional recurrence plots of \((p53 - Mdm2)\), \((p53 - p300)\) and \((Mdm2 - p300)\) are presented in Fig. 4 which provides clear and qualitative picture of the above facts. The emergence of sustain/limit-cycle oscillation (activated p53 level) from fixed point oscillation (stabilized p53 level), and then from sustain oscillation to again fixed point oscillation is observed as one increase the concentration of \(p300\).

Impact of \(p300\) on \(p53-Mdm2\) activation

We first present the deterministic results on p53-Mdm2 regulatory network on exposure to different concentrations of \(p300\) i.e. at different rate constants, \(kp_{300}\) (Fig. 2). For small values of \(kp_{300} (= 0.04)\) (lower \(p300\) concentration), p53 is first activated for some time (\(\sim 30\) hours) and then resumes its normal condition indicated by its constant level (\(\sim 12.4\)) which is the level of stabilization. The range of activation is increased as \(kp_{300}\) increases (increase of \(p300\) concentration) as well as there is rise in the level of stabilization. However, when \(kp_{300} \approx 0.06 - 0.08\), p53 maintains sustain oscillations which leads to increasing level of activation as a consequence.
deacetylates and then ubiquitinates the acetylated p53. Because of this process of interaction of HDAC1 with p53, both p53 and Mdm2 levels get stabilized. In our numerical simulation, we kept p300 concentration level fixed by keeping $k_{p300} \approx 0.08$ throughout the simulation and allow HDAC1 concentration to vary by changing $k_{HDAC1}$ value. The results are shown in Fig. 5 (a)–(f). In these plots we observe that at lower concentration of HDAC1 ($k_{HDAC1} \approx 0.0002$), the p53 activation is large due to pre-existing p300, as indicated by the sustained oscillation (Fig. 5 (f)). This activity suggests that there is regular decay and creation of p53, due to the presence of high levels of p300 and hence the impact of HDAC1 concentration level is not very significant. As the HDAC1 concentration increases (increasing $k_{HDAC1}$ value), there is regular and competitive effect between p300 and HDAC1 for p53 that decreases p53 activation as indicated by decrease in p33 concentration level (Fig. 5 (c)–(e)). Further, if we increase the

Figure 3. Mdm2 dynamics for various p300 levels. The plots of Mdm2 concentration levels as a function of time in hours for various $k_{p300}$ values: (a) $k_{p300} = 0.1$, (b) $k_{p300} = 0.09$, (c) $k_{p300} = 0.08$, (d) $k_{p300} = 0.06$, (e) $k_{p300} = 0.05$ and (f) $k_{p300} = 0.04$ respectively at constant value of $k_{HDAC1} = 0.04$. doi:10.1371/journal.pone.0052736.g003

Figure 5. Activation of p53 via variation of HDAC1 level. Plots of p53 concentration levels as a function of time in hours for various $k_{HDAC1}$ values 0.0002, 0.002, 0.008, 0.01, 0.02 and 0.04 respectively (at constant value of $k_{p300} = 0.08$), showing activation and stabilization of p53. doi:10.1371/journal.pone.0052736.g005

Figure 4. Two-dimensional recurrence plots of p53 and Mdm2. Recurrence plots between (p53 – Mdm2), (p53 – p300) and (Mdm2 – p300) for different values of rate constants $k_{p300}$, i.e. 0.04, 0.05, 0.06, 0.08, 0.09 and 0.1 respectively. doi:10.1371/journal.pone.0052736.g004
concentration of HDAC1, the p53 first activates for short period of time and then remains constant at same value (~50) indicating p53 stabilization. This transition from p53 activation to stabilization is indicated by the transition from sustained oscillation to fixed point oscillations indicated in Fig. 5 (a) and (b). We observe this behaviour at $k_{\text{HDAC}1}=0.04$, where the activity of p53 is low, stable and very much controlled.

Similarly, we present the simulation results of Mdm2 as a function of time for different HDAC1 levels (Fig. 6 (a)–(f)). We observe similar behaviour of Mdm2 as p53 which shows a transition from sustain oscillation to fix point oscillation as one increase the HDAC1 concentration level. These results indicate that HDAC1 stabilizes p53 as well as Mdm2 concentration levels.

We also present the two dimensional recurrence plots of the (p53 − Mdm2), (p53 − HDAC1) and (Mdm2 − HDAC1) for demonstrating these facts (Fig. 7). The clear indication of transition from sustain/limit cycle oscillation to fix point oscillation as $k_{\text{HDAC}1}$ is increased, is shown in the plots indicating transition from activation of p53 and Mdm2 to stabilized state.

**Stability analysis of p53 and Mdm2**

We then checked how concentration level of p53 varies as a function of $k_{p300}$ (i.e. concentration levels of p300). This is done by defining a parameter called exposure time (t) which can be stated as the amount of time the system is exposed to a particular concentration level of p300 or HDAC1. The calculation of p53 or Mdm2 concentration level induced by the exposition of the system to p300 or HDAC1 is done by obtaining its level just after the exposure time (time slice calculation). Fig. 8 shows variation of p53 concentration levels as a function of $k_{p300}$ for different exposure times of 10–100 hours for a fixed value of $k_{\text{HDAC}1}=0.04$. The plots clearly show the activated and stabilized regimes. The activated regime is where the p53 levels fluctuate as a function of $k_{p300}$ (induced by p300 levels). In the plots, p53 level starts activation from $k_{p300}=0.27$ because of the interaction among p53, Mdm2 and p300 with small level of HDAC1 giving rise to fluctuation in p53 level. This could be due to acetylation and deacetylation which leads to capping (which prohibits p53 to decay) and uncapping (which leads to p53 decay) of p53 due to p300. This p53 level fluctuation persists till $k_{p300}=0.55$ and then increases its level without fluctuation till $k_{p300}=2.74$ indicating only the capping of p300, then its level remain constant. Interestingly the range of activation of $k_{p300}$ in p53 for all expose times remain the same in [0.27−2.74].

The stabilized regimes are where p53 level is not affected by the variation in $k_{p300}$ (p300 level variation). Initially, within the range of $k_{p300}$ [0–0.27], the p53 level is not much affected indicating that the cell resumes its normal condition maintaining its minimum level (~13) which we call first stabilization regime. However, in
The first stabilization regime is within \([0–0.23]\) values of \(M_{\text{dm}}\) shown in Fig. 9. Since activated by uncontrolled may be the condition where death of the cell could happen due to uncontrolled \(p_{53}\) growth due to excess \(p_{53}\) concentration level as a function of \(t\). From the plots one can see the activation of \(p_{53}\) at low \(k_{\text{HDAC}}\) value increases, the \(p_{53}\) level starts decreasing due the deacetylation of \(p_{53}\) which in fact is the counterpart of \(M_{\text{dm2}}\) stabilization in our system.

The activation and stabilization of \(M_{\text{dm2}}\) induced by \(p_{300}\) is maximum utilizing \(p_{300}\) which prohibits decay. This case may be the condition where death of the cell could happen due to uncontrolled \(p_{53}\) growth due to excess \(p_{300}\).

The activation and stabilization of \(M_{\text{dm2}}\) induced by \(p_{300}\) is shown in Fig. 9. Since \(M_{\text{dm2}}\) is counter part of \(p_{53}\) which is activated by \(p_{53}\), similar results are obtained as in the case of \(p_{53}\). The first stabilization regime is within \([0–0.23]\) values of \(k_{\text{p300}}\), followed by activation regime \([0.23–0.7]\) and second stabilization regime \([0.7–\infty]\). The increased level of \(p_{53}\) in the second stabilization regime are capped \(p_{53}\) level which are prohibited from decay and taking part in any other reactions and therefore is not able to activate \(M_{\text{dm2}}\) level. Hence its level reduces to minimum as soon as the second stabilization regime is reached.

Next we study the impact of \(H\text{DAC1}\) on \(p_{53}\) stabilization in our system. This is done by keeping the value of \(k_{\text{p300}}\) fixed at 0.08 and simulating the level of \(p_{53}\) as a function of \(k_{\text{HDAC1}}\) for different exposure times 10–100 hours (Fig. 10). From the plots one can see the activation of \(p_{53}\) at low \(k_{\text{HDAC1}}\) values due to \(p_{300}\) impact but not due to \(H\text{DAC1}\) contribution. As \(k_{\text{HDAC1}}\) value increases, the \(p_{53}\) level starts decreasing due the deacetylation of \(p_{53}\) which allow it to degrade and take part in reactions. The activation of \(p_{53}\) with fluctuation persists till \((k_{\text{HDAC1}} \leq 0.1)\). After \((k_{\text{HDAC1}} > 0.1)\), \(p_{53}\) level remains constant for a short period of time and then its level starts increasing without fluctuation. This behaviour indicates that \(H\text{DAC1}\) has suppressing impact on \(p_{53}\) activation. This pattern is same for all exposure times as is shown in the plots (Fig. 10). The same pattern is found for \(M_{\text{dm2}}\) also which in fact is the counterpart of \(p_{53}\). The activated and stabilized regimes are shown in the Fig. 11.

We then present the results of amplitudes of \(p_{53}\), \((A_{p_{53}})\) and time period, \((T_{p_{53}})\) as a function of \(k_{\text{p300}}\) and \(k_{\text{HDAC1}}\) to understand the how \(p_{300}\) and \(H\text{DAC1}\) influence the amplitude and time period of \(p_{53}\) oscillations (Fig. 12). The calculation of \(p_{53}\) amplitude is done as in the following. For sustain oscillation we took time range of [100–200] hours in our calculation and then calculated the average of it. Then we take 50 such time series for different initial conditions and determine the average of \(p_{53}\) amplitude again (Fig. 12 and 13). The points in the plots are average points with error bars. For damped oscillations, we take the available number of oscillations and calculated the average of those oscillations which is found to be equivalent to the distance between x-axis and line which shows no oscillation (stable line) approximately. Similarly, for stabilized regime we determine

\[
\text{Figure 8. Stability curve induced by } p_{300}. \text{ Plots of } M_{\text{dm2}} \text{ concentration level as a function of } k_{\text{p300}} \text{ for different values of exposure times i.e. } \eta = 10–100 \text{ (at constant value of } k_{\text{HDAC1}} = 0.04) \text{. In the curve stabilized and activated regimes are demarcated.} \hspace{1cm} \text{doi:10.1371/journal.pone.0052736.g008}
\]

\[
\text{Figure 9. Stability curve induced by } p_{300}. \text{ Plots of } M_{\text{dm2}} \text{ concentration level as a function of } k_{\text{p300}} \text{ for different values of exposure times i.e. } \eta = 10–100 \text{ (at constant value of } k_{\text{HDAC1}} = 0.04) \text{. In the curve stabilized and activated regimes are demarcated.} \hspace{1cm} \text{doi:10.1371/journal.pone.0052736.g009}
\]

\[
\text{Figure 10. Stability curve induced by } H\text{DAC1}. \text{ The variation of } p_{53} \text{ concentration level versus } k_{\text{HDAC1}} \text{ for different exposure times } \eta = 10–100, \text{ keeping } k_{\text{p300}} = 0.08 \text{ fixed. The inset is the enlarged portion of the actively activated regime.} \hspace{1cm} \text{doi:10.1371/journal.pone.0052736.g010}
\]

\[
\text{Figure 11. Stability curve induced by } H\text{DAC1}. \text{ The variation of } M_{\text{dm2}} \text{ concentration level versus } k_{\text{HDAC1}} \text{ for different exposure times } \eta = 10–100, \text{ keeping } k_{\text{p300}} = 0.08 \text{ fixed. The inset is the enlarged portion of the actively activated and stabilized regimes.} \hspace{1cm} \text{doi:10.1371/journal.pone.0052736.g011}
\]
distance between x-axis and stable line for each values of $k_{p300}$ or $k_{HDAC1}$ and average over 50 time series. Initially, $A_{p53}$ remains constant at lowest value for small values [0–0.05] of $p$ and monotonically increases and decrease in the interval [0.05–0.3] and finally its value remains constant. This in fact is the consequence of first stability (normal condition) where the impact of $p300$ is negligible, then activation of $p53$ due to interaction of $p300$ with $p53$ and other proteins and then stabilization of $p53$.

These three regimes can also be seen in the case of $T_{p300}$ versus $k_{300}$ plot.

However, in the case of $A_{p53}$ and $T_{p53}$ induced by $HDAC1$, the first stability condition is not observed because the cell is already activated with a constant $p300$ level i.e. at constant $k_{p300}=0.08$. In this case $A_{p53}$ level decreases as $k_{HDAC1}$ increases till $k_{HDAC1}=0.03$ and the remains constant. However, $T_{p53}$ increases till $k_{HDAC1}=0.03$ and then stabilized.

Similarly, we calculated $A_{Mdm2}$ and $T_{Mdm2}$ as a function of $k_{300}$ and $k_{HDAC1}$ respectively and the results are shown in Fig. 12. For both the parameters similar behaviour was obtained as in the case of $p53$.

**Deterministic steady state solutions: impact of $HDAC1$ and $p300$ on $p53$**

The steady state solutions in deterministic case can be obtained by putting the conditions $\frac{dx_i(t)}{dt} = 0$, $i = 1, 2, \ldots, N$, where $N = 14$, to the set of differential equations (3)–(16) and solving for various variables $\{x'_i\}$. Following this procedure we first solve for $x_{11}^{st}$ (steady state solution of $p53$) as a function of $x_{11}$ (steady state solution of $HDAC1$). The result is given by,

$$x_{11}^{st}(x_{11}) \sim \Gamma \left( z + \beta x_{11}^{*} + \frac{\gamma}{x_{11}} \right) \quad (31)$$

where, $\Gamma = \frac{k_{14}}{k_{18} k_{22}}$, $x = k_{23}(1 + \frac{k_{14} k_{18}}{k_{22} k_{23}}) - \frac{k_{22} k_{24}}{k_{18}} (k_{3} + \frac{k_{18}}{k_{22}})$, $\beta = k_{19}(1 + \frac{k_{22}}{k_{18}} + \frac{k_{14}}{k_{20}})$ and $\gamma = k_{24} - \frac{k_{22} k_{23}}{k_{18}} - \frac{k_{24} k_{23}}{k_{20}}$. These are constants. The equation (31) shows that the increase in $x_{11}$ leads to increase in $x_{11}^{st}$ and second term in the equation is the main contributor. The reason being as $x_{11}$ increases the third term $\frac{1}{x_{11}} \rightarrow 0$ and the first term is a constant. Further, increase in $k_{22}$ and $x_{11}$ (degradation rate of $HDAC1$) and $k_{14}$ (degradation rate of $p300$) contribute increase in $\beta$, and therefore increases the steady state level of $x_{11}^{st}$. From the expression of $\beta$, one can see that $\frac{k_{23}}{k_{18}} > 1$ ($p300$ synthesis rate is larger than $HDAC1$ degradation rate), $\beta$ will contribute positive to $x_{11}^{st}$, otherwise it will give negative contribution.

Proceeding in the same way, the steady state solution of $x_{21}^{st}$ ($Mdm2$) can be obtained as a function of $x_{11}$. The result is given by,

$$x_{21}^{st}(x_{11}) \sim \rho \left( \frac{\epsilon}{x_{11}} - 1 \right) \quad (32)$$

where, $\rho = \frac{k_{22}}{k_{18}}$ and $\epsilon = \frac{k_{24}}{k_{22}}$. These are constants. It can also be seen from the equation (32) that $x_{21}^{st} \propto k_{22}$ ($k_{22}$ is degradation rate of $HDAC1$). Further for positive $x_{21}^{st}$, we have the condition $x_{11}^{st} \times \epsilon = \frac{k_{24}}{k_{22}}$ which means that the creation rate of $HDAC1$ ($k_{24}$) should be larger than degradation rate of $HDAC1$ ($k_{22}$) provided the condition. This behaviours can be seen in Fig. 8.
Next we solve for steady state solution \( x_2^{ed} \) of \( Mdm2 \) as a function of \( x_1^* \) (steady state solution of p300) to study the impact of p300 on Mdm2. The result is given by,

\[
x_2^{ed} (x_1^*) = \frac{a - x_1^*}{x_1^* - b}
\]

(33)

where, 
\[
c = \frac{k_{22}k_{18}}{k_{20}k_{22} + k_{18}}, \quad ha, k_{23}, b = \frac{k_{23} - k_{18}k_{24}}{k_{24} + k_{20}k_{22}} \quad \text{are constants.}
\]

From equation (33) for positive \( x_2^{ed} \) one can either \( a < x_1^* \) and \( x_2^{ed} > b \) or \( a > x_1^* \) and \( x_2^{ed} < b \). Moreover b to be positive the condition \( k_{23} > k_{21} \) should be satisfied.

Now we solve steady state solution of \( x_1^{ed} \) as a function of \( x_1^* \) to understand the impact of p300 on p53. The result is given by,

\[
x_1^{ed} (x_1^*) = x_2^{ed} (x_1^*) (u + x_1^* + \frac{v}{w + x_2^{ed} (x_1^*)})
\]

(34)

where, \( s = \frac{k_{21}k_{18}}{k_{20}k_{31}}, \quad u = \frac{k_{25}}{k_{20}}, \quad v = \frac{k_{24}}{k_{20}} \quad \text{and} \quad w = \frac{k_{22}}{k_{18}} \quad \text{are constants.}
\]

\( x_1^{ed} (x_1^*) \) is given by the equation (33). The equation (34) indicates that \( x_1^{ed} \) is increased by increase in \( x_1^* \) but decrease in \( x_2^{ed} \). Further if \( k_{24} \), the synthesis rate of HDAC1 is increased then \( x_1^{ed} \) will also be increased. It can also be seen from \( s \) and (34) that \( x_1^{ed} \propto \frac{1}{k_2} \) (synthesis rate of Mdm2).

The role of noise and stabilization on p53 – Mdm2 regulation

Now we present the role of noise on p53 and Mdm2 dynamics. This is done by solving the CLE equations (16)-(29) numerically. The results for different system size parameter, \( V \) (1-50) at constant values of \( k_{p300} \) and \( k_{HDAC1} \), are shown in Fig. 14 (a)-(f). It has been observed that for \( V = 1 \), no oscillation in p53 is seen. However, as \( V \) increases the oscillation starts emerging and when \( V = 25 \) and 50 sustained oscillations are observed with increasing p53 level. After \( V = 50 \) i.e. for \( V \geq 50 \), the p53 level remains constant i.e. it exhibits sustained oscillatory behaviour. The p53 dynamics is noise induced stochastic process and the strength of noise decreases as \( V \) increases. The same behaviour is also seen in Mdm2 dynamics keeping all conditions the same (Fig. 14 (a)-(f)).

Now we present the impact of p300 on p53 and Mdm2 in stochastic system by simulating p53 and Mdm2 levels as a function of \( k_{p300} \) for different \( V \) (Fig. 15). The result for \( V = 10 \) shows similar pattern as we found in the deterministic case, but the two conditions of stabilization and activation is achieved earlier with respect to \( k_{p300} \) in stochastic case than that of the deterministic case as shown in the insets of the Fig. 15. Further, as one increases \( V \), the values \( k_{p300} \) for getting the two conditions of stabilization and activation are increased.

The dynamics of p53 concentration remains constant with small fluctuations around the constant values of \( V \sim 1 - 15 \) even though there is a small damping behavior at initial few hours. We then define \( T_V \) as the critical time below which the dynamics either shows damped or fixed point (stabilized) oscillations. The plot (\( T_V - V \)) in Fig. 16 shows the damped, stabilized and oscillatory regimes. To generate this plot we took 50 simulations for a certain fixed set of parameters and points in the curves show average values with error bars which are correct up to the order

![Figure 14. Noise contribution on p53 dynamics in stochastic system.](image-url)
of 5-10 percent in our calculation as shown in Fig. 16. The plots show how system size, which can be taken as noise parameter (as V increases noise strength decreases and vice versa), drives the system at different states, namely, damped, stabilized (no oscillation) and sustain oscillation regimes.

We also study the impact of exposure time (\(t\)) on p53 activation and stabilization for different values of V keeping the value of \(\eta\) constant. We can see from the two left panels with insets in Fig. 15 that as \(\eta\) increases the conditions of stabilization and activation are obtained faster.

The results showing the impact of p300 on p53 in stochastic system for different V’s and \(\eta\)s are presented in Fig. 17. We also get the similar behaviour in the case as obtained in the case of Mdm2 as shown in Fig. 18.

**Stochastic steady state solutions: the noise effect**

The steady state solutions of CLE can also be obtained as we did in deterministic case from the equations (17)–(30). We first impose steady state condition to the set of CLEs i.e. \(dx_i/dt = 0\) and got a set of steady state equations which are very difficult to solve. However, the steady state solutions can be obtained if we neglect negligible terms which have \(O(\xi^2)\) and \(O(V^{-3})\) and rearrange the terms to solve the equations. Then one can easily solve simplified steady state equations. Proceeding in this way, the stochastic steady state solution of \(x_i^d\) as a function of time \(t\), can be obtained given by,

\[
x_i^d(x_i^s, V, \xi) \sim x_i^d(x_i^s) + A(x_i^s, V) \xi,
\]

where, \(x_i^d(x_i^s)\) is given by equation (31) and we have taken the noise parameters \(\{\xi\}\) associated with each noise term are taken to be the same as \(\xi_s\). The noise term \(A(x_i^s, V)\) is given by,

\[
A(x_i^s, V) \sim \frac{A}{\sqrt{V}} \left[ (q_1 - q_2 \sqrt{\epsilon - x_i^s}) \left(1 - \frac{q_3}{x_i^s}\right) + \sqrt{k_2 \rho \left( \frac{\epsilon}{x_i^s} - 1 \right)} \right]
\]

\[
+ \frac{A}{\sqrt{V}} \left[ \sqrt{k_{18} \rho (\epsilon - x_i^s)} + q_4 \left(1 + q_4 x_i^s + \frac{q_6}{x_i^s}\right) \right]
\]

where, \(A = \frac{k_4}{k_3 k_1}, q_1 = \frac{k_{2k_{20}} \sqrt{k_{23}}}{k_{14} - k_{20} \rho}, q_2 = \frac{k_{2k_{20}} \sqrt{k_{18} \rho}}{k_{14} - k_{20} \rho}, q_3 = \frac{k_{20} \rho c}{k_{14} - k_{20} \rho}, q_4 = \frac{k_{23} - k_{18} \rho}{1 + \frac{k_{14}}{2k_{20} \rho} + \frac{k_{14k_{18}}}{2k_{20}(k_{23} - k_{18} \rho)}}\),

\[
q_5 = \frac{\left(1 + \frac{k_{14}}{2k_{20} \rho}\right) \frac{k_{18} \rho}{k_{23} - k_{18} \rho}}{1 + \frac{k_{14}}{2k_{20} \rho} + \frac{k_{14k_{18}}}{2k_{20}(k_{23} - k_{18} \rho)}},
\]

\[
q_6 = \frac{k_{14f}}{2k_{20} \rho \left[1 + \frac{k_{14}}{2k_{20} \rho} + \frac{k_{14k_{18} \rho}}{2k_{20}(k_{23} - k_{18} \rho)}\right]},
\]

are constants. It
Similarly, the stochastic steady state solutions of $x_1^s$ and $x_2^s$ as a function of $x_1^p$ along with their respective deterministic solutions given by equations (33) and (34) can also be calculated. The results are given by,

$$x_1^s(x_1^p) \sim x_2^s(x_1^p) + \chi(x_1^p, V) \zeta_k$$

and

$$x_2^s(x_1^p) \sim x_2^d(x_1^p) \left[ 1 - \frac{h}{x_3^p - b \sqrt{V}} \right]$$

where, $\zeta_k$ and $\zeta_d$ are random noise parameters for equations (39) and (40) respectively. The function $\chi$ is given by

$$\chi(x_1^p, V) \sim \frac{1}{\sqrt{V}} \left[ \frac{h x_3^d}{x_3^p - b} \left( \frac{\lambda x_3^d}{w + x_2} - k_20 x_3^p - k_s \right) + \frac{\eta}{w + x_2^d} \right]$$

$$+ \frac{\phi}{\sqrt{V}} \left( \sqrt{x_2^d} + \sqrt{k_20 x_3^p} + \sqrt{k_3} \right)$$

where, $h = k_1 \sqrt{k_{24}}$, $\lambda = k_{18} k_{24}$, $\eta = k_{24} + \sqrt{k_{24}}$ and $\phi = \frac{k_4}{k_{24}}$ are constants. The noise function $\chi$ is mainly contributed from first, fifth and sixth terms in equation (41) and $\chi$ is positive contributor to the deterministic part. From these main contrib-

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**Figure 17. Stabilization of p53 in stochastic system.** (a) Plots of p53 concentration levels as a function of $p300$ for different values of system size, $V = 10, 30, 50$ and 70 and for different values of $\eta = 10$ to 100 as shown in the four left panels. The insets show the enlarged portions of the activated regimes in each case. (b) Plots of $p53$ level versus $k_{p300}$ for different $V = 10, 30, 50, 70$ and for two different values of $\eta = 10$ and 100 respectively as shown in two right hand panels.

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can be seen from equation (36) that the terms apart from first term and last terms in the last parenthesis will contribute to $A$ only when $x_1^p < \lambda$. Hence for $x_1^p < \lambda$, the equation (36) will have the following expression,

$$A(x_1^p, V) \sim \frac{A}{\sqrt{V}} \left[ e + f x_1^p + \frac{g}{x_1^p} \right]$$

where, $e = q_1 + q_2$, $f = q_4 q_5$ and $g = q_4 q_6 - q_1 q_3$. It can also be seen from $A$ and equation (36)-(37) that $A \propto \frac{1}{k_3}$.

Next we calculated the steady state solution of $x_2^d$ as a function of $x_1^p$. The result can be expressed along with the deterministic result as shown in equation (32) with noise term. It is given by,

$$x_2^d(x_1^p) \sim \frac{1}{2} x_2^d(x_1^p) - \frac{\zeta_d}{4 k_{18} \sqrt{V}} \left( \sqrt{k_{22}} \frac{1}{x_1^p} + \sqrt{k_{24}} \frac{1}{x_1^p} \right)$$

where, $\zeta_d$ is the random noise parameter which we have taken same for all terms involved in the derivation. The noise contribution in this case is negative to the deterministic result which reduces steady state level of $x_2^d$ as the strength of noise increases. Further the increase in degradation and synthesis rate of HDAC1 ($k_{22}$) lead to increase in noise contribution which in turn decreases $x_2^d$.

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**Figure 18. Stabilization of Mdm2 in stochastic system.** (a) Plots of Mdm2 concentration levels as a function of $p300$ for different values of system size, $V = 10, 30, 50$ and 70 and for different values of $\eta = [10-100]$ as shown in the four left panels. The insets show the enlarged portions of the activated regimes in each case. (b) Plots of Mdm2 level versus $k_{p300}$ for different $V = 10, 30, 50, 70$ and for two different values of $\eta = 10$ and 100 respectively as shown in two right hand panels.

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ing terms, the synthesis rate of HDAC1, $x_{2}^{ed}$ and $x_{8}$ and their variation give significant contributions to the noise terms in equations (39) and (40). However noise contribution in equation (40) is negative contributor to the deterministic part.

**Conclusion**

The interaction of p300 with p53 allows p53 to be acetylated which prohibits it from decaying and allows it to participate in other reactions. This excess in p300 level eventually leads to increase in capped p53 whose population cannot be controlled and subjects the cell to stress condition. If the excess in p300 level is strong enough it may lead to cell death due to uncontrolled p53, similar to cancer. We observe this phenomena in our simulation results in qualitative sense via three different stages/conditions, namely, first stabilization or normal condition where impact of $p_{53}$ is negligible, second activation of p53 due to significant interaction between p300 and p53, and third uncontrolled growth of capped p53 due to interaction with excess p300 leading to second stabilization level which may represent cell death condition. The same behaviour is seen in Mdm2 simulation results. The three conditions of stabilization and activation are obtained but the second stabilization level is obtained at lower level as compared to first stabilization level. This may be due to the fact that the increase of capped p53 cannot activate Mdm2 as is done normally, and goes to lower minimum level.

The interaction of HDAC1 with p53 will cause deacetylation of capped p53 which leads p53 to participate in other reactions and able to decay. This may help the already stressed cell to bring back to its normal condition. However excess of HDAC1 will cause excess deacetylation of p53 and will allow the cell to come back far beyond to its normal condition leading to stress. Our results supports these findings.

Noise has interesting but contrasting roles in stochastic system depending upon its strength. If its strength is strong then it has destructive impact on the signal processing in and outside the system etc. However if its strength is weak then it exhibit constructive role, for example weak signal detection, amplification and processing the signal etc. In our study, we found that if the system size is very small where the noise strength is very strong with respect to system size, the associated noise destroy the signal in the system which is in agreement with the theoretical claim. But if the system size is increased in our study where noise strength is comparatively weaker, the signal is resumed in normal with noise induced dynamics in each variable. Moreover, in stochastic system, the p53/ Mdm2 is activated by small concentration level of p300/HDAC1 as compared to those in deterministic case and reach stabilization much much faster as compared to deterministic system. Further increase in system size reduces the noise fluctuation in the dynamics of each variable and when $V \rightarrow \infty$, the noise strength is negligible and the system goes to classical deterministic system.

In the present study we determine only the impact of p300 and HDAC1 on p53—Mdm2 regulatory network. For developing any realistic model one needs to incorporate other proteins which influence p53 protein simultaneously and then study the impact collectively. Our study is just one step forward towards understanding p53 regulatory network.

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**Author Contributions**

Conceived and designed the experiments: RKBS SMA. Constructed the model and performed the simulation: AA SG TM DR MJA RKBS SMA. Did the analysis of the simulation: AA SG TM DR MJA RKBS SMA. Wrote the paper: AA SG TM DR MJA RKBS SMA.

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