

## Fine tuning of p53-Mdm2-MdmX network by MdmX during DNA damage response

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Tumor suppressor protein p53 is a transcription factor regulated by two structurally homologous proteins, Mdm2 and MdmX. Mdm2 is a ubiquitin ligase that targets p53 for degradation. In contrast, MdmX lacks ubiquitin ligase activity and the mechanism of its regulation of p53 is not well understood. In some experimental studies, MdmX has been reported either as a stabilizer<sup>1</sup> and activator<sup>2</sup>, or as a negative regulator<sup>3</sup> of p53. DNA damage activates a phosphorylation cascade that alters the binding interactions between p53, Mdm2 and MdmX, leading to the stabilization and activation of p53. In this study, we investigated interaction models consisting of p53, Mdm2 and MdmX to elucidate how those two proteins interact to regulate the p53 activity.

We have assembled the known molecular interactions among p53, Mdm2, and MdmX to generate a molecular interaction map (figure 1) using the previously described notation<sup>4</sup>. The map was used to generate ODEs based on mass action laws. DNA damage was simulated as an increase in the phosphorylation rates of p53, Mdm2, and MdmX. Since p53 acts as a transcription factor, the concentration of a promoter-bound p53 tetramer (species 14 in figure 1) was used as a measure of p53 response in the simulations.

Simulation results show that during the early response to DNA damage (before p53 activates transcription of Mdm2, which in turn leads to a negative feedback loop) MdmX acted as an amplifier of p53 activity, inducing a switch-like response (figure 1). That switch-like behavior of p53 activity resulted from the interplay between two reservoirs, p53:MdmX and Mdm2:MdmX. When Mdm2 levels were low, the p53:MdmX reservoir served as a p53 reservoir, enhancing the availability and activity of p53. When levels of Mdm2 were high, the Mdm2:MdmX complex served as an Mdm2 reservoir, reducing p53 activity. The amplification response was effective transiently until these two reservoirs relaxed into new steady states.

Late in the DNA damage response, p53 transcriptionally activates Mdm2, creating a negative feedback loop. When this loop was included in the models, p53 activity displayed sustained oscillations, consistent with previous studies<sup>5</sup>. Oscillations were observed when the p53-Mdm2 negative feedback loop included a time delay or when the model included a TR3 mediated p53 dependent Mdm2 degradation<sup>6</sup> (*k14*). In oscillating systems with an unstable steady state, MdmX either reduced the amplitude of oscillating peaks or dampened the oscillations (figure 2). In non-oscillating systems, the effects of MdmX were transient and varied (figure 2).

Taken together, our observations illustrate a new mechanism of a biological switch which operates transiently in response to cellular events such as DNA damage and suggest a mechanism for MdmX – mediated modulation of p53 activity.

\* This research was supported in part by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institute of Health

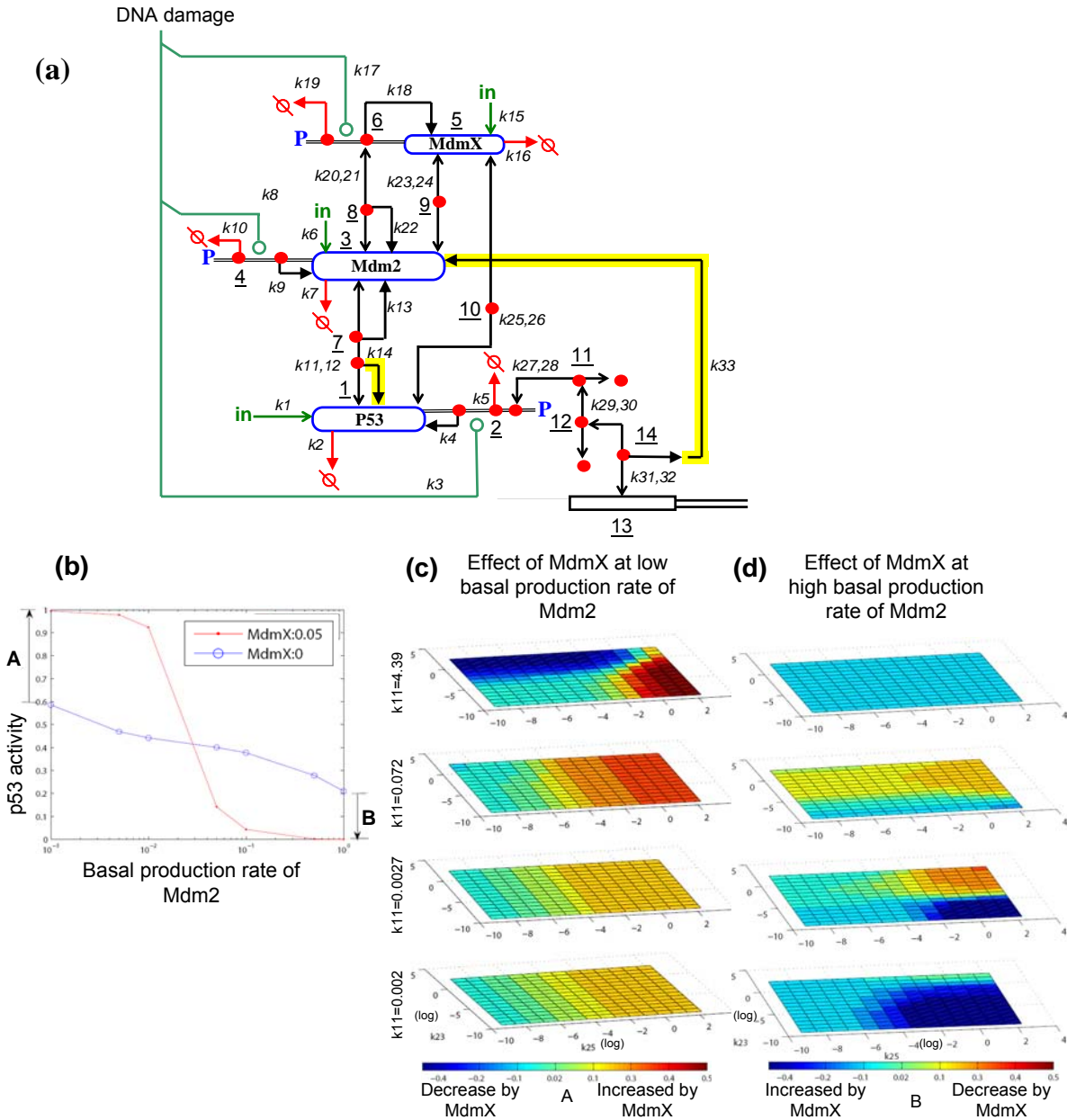


Figure 1. (a) Molecular interaction map of p53-Mdm2-MdmX network. Yellow highlighted lines (k13 and k14) were included only in the late response simulations. (b) Effects of MdmX on p53 activity as a function of basal production rate of Mdm2. It was assumed that the system was at its steady state at the time of DNA damage. The switch like behavior (dots) in figure (b) was generated by the increased p53 activity (A) by MdmX when Mdm2 was low and the decreased p53 activity (B) by MdmX when Mdm2 was high. The effects of MdmX (A and B) in wide range of parameter space were visualized in (c) and (d). (c, d) Left-right axis: MdmX-p53 binding association constant ( $k_{25}$ ); forward-back axis: Mdm2-MdmX binding rate constant ( $k_{23}$ ); vertical axis: p53-Mdm2 binding rate constant ( $k_{11}$ ) (the dissociation rate constants were unchanged).

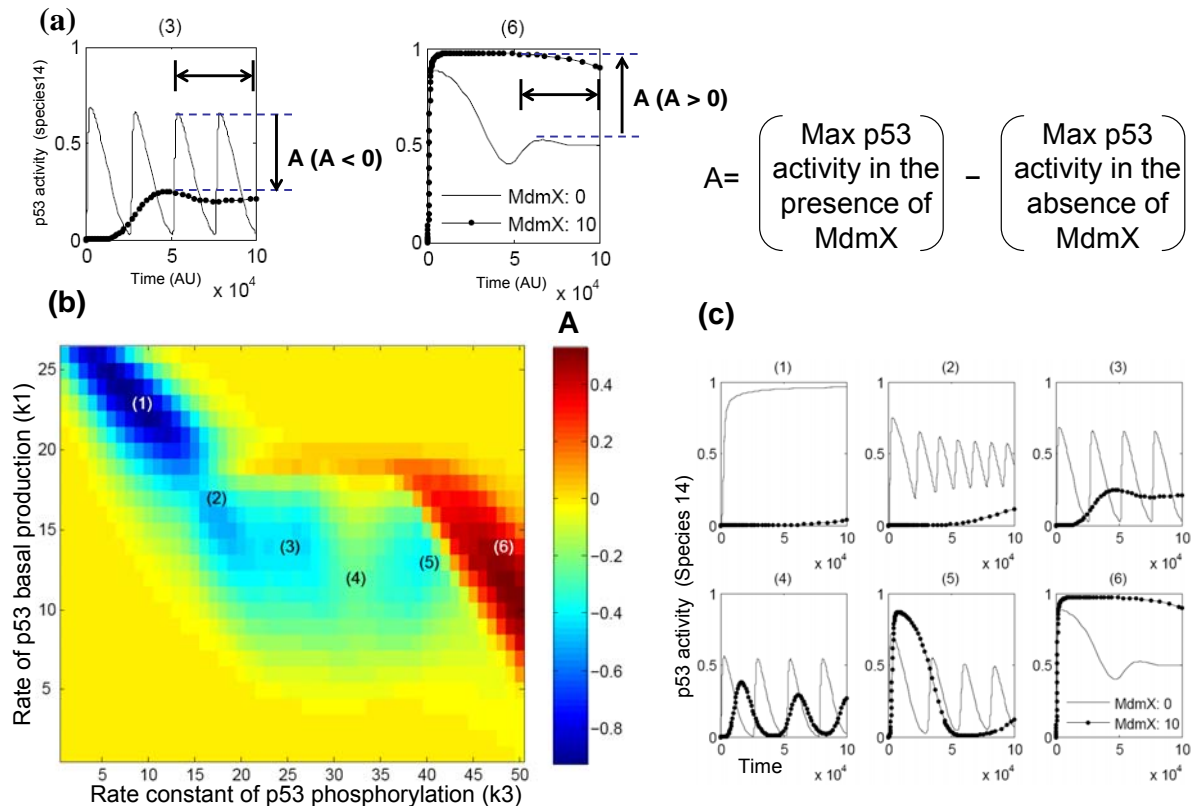


Figure 2. Effects of MdmX on p53 activity during the late response. (a)  $A$  value represents the difference between maximum values achieved during the last half of time series. (b) Color represents the  $A$  value obtained from a pair of simulations. Blue area represents the negative effects of MdmX (c-2~5). Red area represents the positive effects of MdmX (b-6). In oscillating systems, MdmX functioned as a negative regulator by reducing the height of oscillating peaks or by dampening oscillations (c-2~5). In the systems with a stable steady state, MdmX either transiently reduced or elevated p53 activity (c-1, 6), and the effects of MdmX on p53 activity is either negative (c-1) or positive (c-6).

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