

Recurrent Initiation: A Mechanism for Triggering p53 Pulses in Response to DNA Damage

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A major goal of systems biology is to understand the control of signaling pathways in complex networks. We focus on the p53 signaling pathway, one of the most important pathways protecting organisms from developing cancer. Our previous single cell studies showed that p53 is expressed in a series of discrete pulses that vary in number from cell to cell (Lahav *et al.*, 2004). The mean height and duration of each pulse were fixed, and did not depend on the amount of DNA damage. The number of cells showing repeated pulses, however, increased with DNA damage (Geva-Zatorsky *et al.*, 2006). Although a great deal is known about the network of interactions surrounding p53, very little is known about which of these interactions contribute to the dynamical behavior of the system. The simplest explanation consistent with the network structure is the idea that these pulses are oscillations intrinsic to the p53/Mdm2 negative feedback loop.

To clarify the origin of the p53 pulses, we collected dynamic information about several components of the p53 signaling pathway at the population and single-cell level. We found that the upstream checkpoint kinases ATM and Chk2 also show pulses of activity (**Figure 1**) that are required for p53 pulses and, surprisingly, are themselves controlled by p53. A single pulse of activated ATM is sufficient to initiate a single full p53 pulse, but does not trigger multiple p53 pulses. Thus, although the p53-Mdm2 negative feedback loop is clearly important in shaping the dynamics of the system, it is not the only source of the repeated pulses. Instead, combining computational (**Figure 2**) and experimental approaches, we identified a second feedback loop from p53 to ATM, mediated by the phosphatase Wip1, as important for maintaining the uniform pulses of p53 in response to various levels of DNA damage. We propose that the p53 pulses depend on repeated pulses of activated ATM, which is re-activated by persistent DNA damage and deactivated by the Wip1 negative feedback loop.

References

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Geva-Zatorsky, N., Rosenfeld, N., Itzkovitz, S., Milo, R., Sigal, A., Dekel, E., Yarnitzky, T., Liron, Y., Polak, P., Lahav, G., and Alon, U. (2006) *Mol Syst Biol.* 2: 2006.0033.

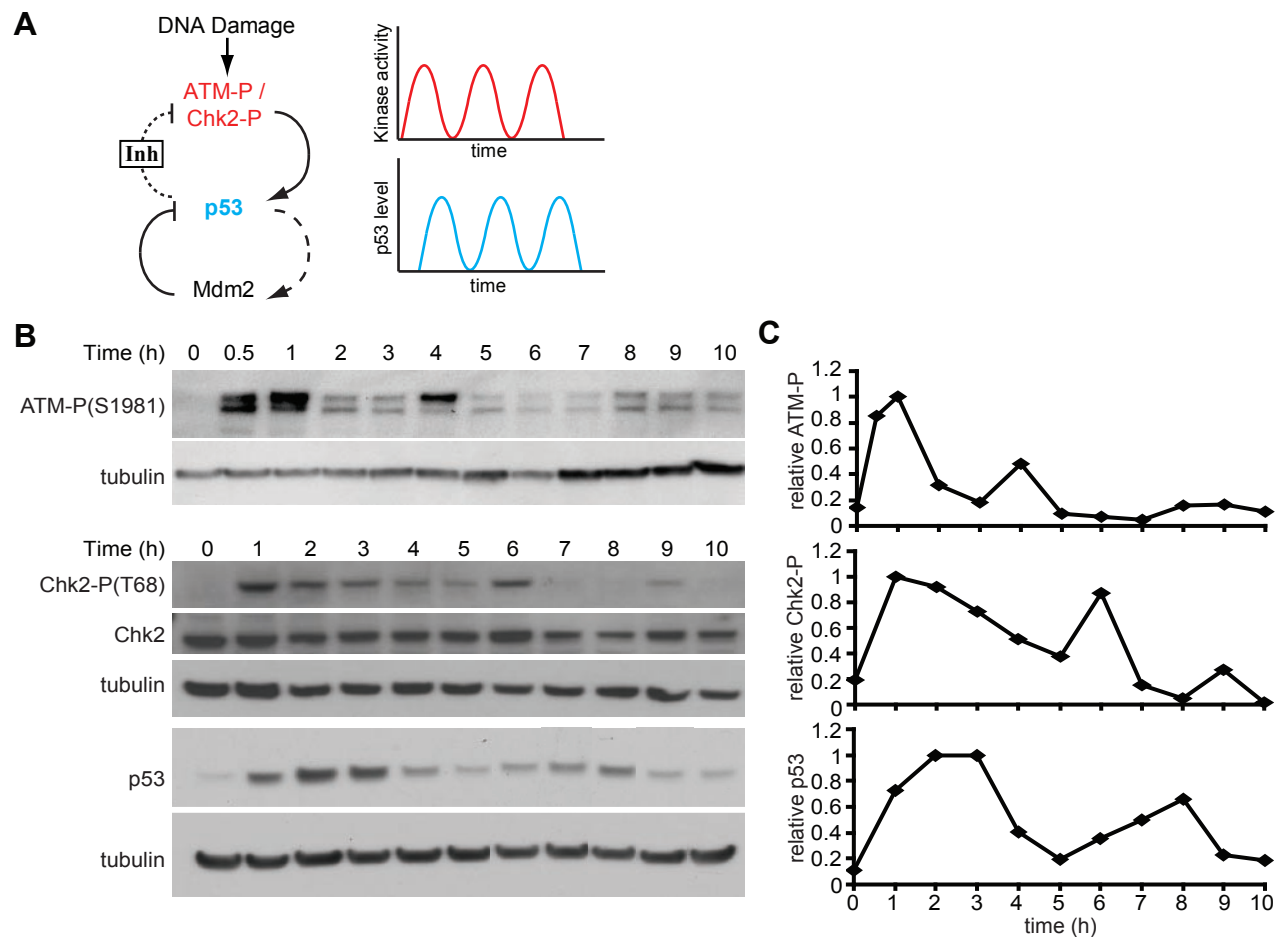


Figure 1: Dynamics of DNA damage kinases. (A) A recent model (Geva-Zatorsky *et al.*, 2006) proposes that in addition to the p53-Mdm2 negative feedback loop p53 creates a separate negative feedback loop with the upstream kinases ATM and Chk2 by upregulating a putative inhibitor “Inh.” In this proposal, the signaling kinase activities may oscillate as well. The nature and rate of feedback between p53 and ATM through the inhibitor “Inh” is unknown. (B) Immunoblots of active ATM (ATM-P(S1981)), active Chk2 (Chk2-P(T68)), and p53 kinetics in MCF7 cells irradiated with 10Gy of γ -irradiation. (C) Quantification of protein levels of blots in (B) normalized by tubulin, in units relative to peak intensity.

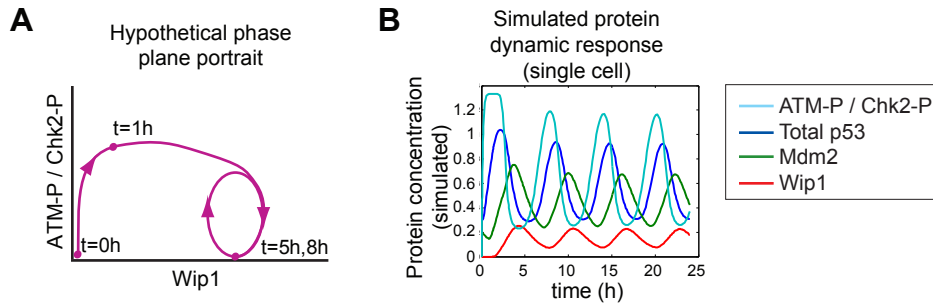


Figure 2: Simulation of the p53 signaling pathway. (A) Hypothetical phase-plane trajectories of concentrations of the kinases ATM-P and Chk2-P and the phosphatase Wip1 following γ -irradiation based on experimental observations. (B) Numerical simulations of the pathway in response to DNA damage. ATM-P / Chk2-P (light blue), total p53 (dark blue), Mdm2 (green), and the inhibitor Wip1 (red) concentrations following γ -irradiation