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

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***Short Abstract* — DNA damage initiates a series of p53 pulses. Although much is known about interactions surrounding p53, little is known about which interactions regulate p53's dynamical behavior. We found that the upstream kinases ATM and Chk2 also show pulses of activity that are required for and are controlled by p53 pulses. Combining experimental and computational approaches, we identified a feedback from p53 to ATM, mediated by the phosphatase Wip1, as important for maintaining the uniformity of p53 pulses. We propose that p53 pulses depend on repeated pulses of activated ATM, which is re-activated by persistent DNA damage and deactivated by Wip1.**

I. Introduction

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, in response to DNA damage induced by gamma irradiation, p53 is expressed in a series of discrete pulses that vary in number from cell to cell [1]. The mean amplitude and duration of each pulse are fixed, and do not depend on the amount of DNA damage. The number of cells showing repeated pulses, however, increases with DNA damage [2]. 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 performed quantitative population and single cell measurements of the dynamics of several proteins in the p53 signaling pathway.

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II. Results

A. The DNA damage signaling kinases ATM and Chk2 show pulses of activity in response to gamma irradiation

The kinases ATM and Chk2 become activated in response to DNA damage. Upon activation, these kinases phosphorylate p53 and Mdm2, leading to the accumulation of p53. By measuring the activation of ATM and Chk2 with high temporal resolution, we found that activated ATM and Chk2 show pulsatile dynamics. Individual pulses of ATM and Chk2 precede p53 pulses, and occur at comparable pulse frequencies.

B. p53 dynamics depend on ATM and Chk2 dynamics

To determine the relationship between the dynamics of signaling network components, we perturbed the system using small molecule inhibitors, RNAi, and radiomimetic drugs. We found that p53 pulses directly depend on ATM and Chk2 pulses, and are not intrinsic to the p53/Mdm2 loop. We also found that ATM and Chk2 pulses depend on p53 pulses, suggesting that a negative feedback between p53 and the upstream kinases may be important for regulating the network's dynamics.

C. The negative feedback loop mediated by Wip1 is important for shaping the network's dynamics

We assembled our data into a theoretical framework and used the result to experimentally identify Wip1 as the mediator of the crucial negative feedback. We also showed that Wip1 plays an important role in regulating both the amplitude and the duration of p53 pulses.

III. Conclusion

We propose that p53 pulses depend on repeated pulses of activated ATM, which is reactivated by persistent DNA damage and de-activated by Wip1.

References

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