Dynamics of p53 in non-stressed conditions

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Short Abstract — The tumor suppressor protein p53, a key player in DNA damage signaling, is regulated by various feedback loops. The architecture of these feedbacks shapes the dynamic response of the pathway. Ionizing radiation, for instance, induces periodic p53 pulses. Here we focused on investigating p53 dynamics in the absence of exogenous stimuli and detected pulses even in non-stressed conditions. Since the timing of these pulses vary between cells, they are detected only by measuring the dynamics of single cells. We characterized the p53 pulses in non-stressed conditions and examined their sources and function.

***Keywords* — Protein dynamics, signaling, p53, DNA damage, single cell experiments, time-lapse microscopy**

I. Purpose

The tumor suppressor p53 is a central player in the signaling network guarding the integrity of our genome. The p53 network responds to cellular stress, ranging from DNA damage to oncogenic transformation, and triggers cell cycle arrest, DNA repair, or apoptosis [1]. Upstream kinases respond to stress by phosphorylating p53, thereby stabilizing the protein and leading to its accumulation. Various feedback loops, including the ubiquitin ligase Mdm2 and the phosphatase WIP1, counteract p53 activation [2, 3]. The architecture of these feedback loops shapes the dynamic response of the pathway.

Previous work on p53 focused on population studies averaging the dynamics of millions of cells. Our lab focuses on studying individual cells, using time-lapse microscopy to measure p53 levels in living cells challenged with different types and doses of DNA damage. For example, we have found that in response to irradiation, p53 levels show a series of undamped pulses [4, 5]. It is, however, unknown what the dynamic behavior of p53 is in the absence of exogenous stimuli. Although p53 is believed to be kept at low levels under these conditions, the activation threshold for p53 may be low, leading to frequent firing of the pathway even in the absence of external stress. In this study we focused on characterizing p53 dynamics in non-stressed conditions and investigating their mechanism and function.

II. Experimental Approach and Results

To measure p53 dynamics in non-stressed conditions we fused p53 to Venus, a fast maturating fluorescent protein and used fluorescence microscopy to follow p53 dynamics in irradiated and non-irradiated living cells. We used a pulse detection algorithm to identify the p53 pulses in single cell trajectories and evaluated their characteristics. As previously observed, ionizing radiation triggered periodic uniform pulses of p53 [4]. Surprisingly, with no external stress, single cells showed pulses of p53 as well. While irradiation triggered an immediate series of pulses in all cells, pulses in unstressed cells were less frequent and non-synchronous.

We next asked whether p53 pulses in non-stressed conditions result from random noise in the network or whether they are triggered in response to internal DNA damage. Using siRNA and small molecule inhibitors, we found that the same upstream kinases that activate p53 in response to irradiation elicit p53 pulses in non-stressed conditions, suggesting that they result from spontaneous DNA damage or replicative stress. To address that we developed a system that allows measurement of both p53 levels and DNA double strand breaks in individual living cells over time. With such a system, we are able to measure whether there is a threshold of DNA damage, caused by external or internal sources, above which the p53 network triggers a p53 pulse.

Furthermore, we investigated whether p53 induction in non-stressed condition leads to activation of its target genes. Using a fluorescent reporter, we monitored the expression of the canonical target gene p21 in irradiated and unstressed cells. Interestingly, we observed induction of p21 after p53 pulses only upon irradiation, suggesting that the signaling network is able to filter p53 activation in unstressed cells.

III. Conclusion

Taken together, our results show that the p53 network is more dynamic than previously recognized and frequently activated even in unstressed cells. This observation emphasizes the importance of our experimental approach and revealed how complex the p53 signaling network is; in response to DNA damage p53 does not shift from a quiet mode into pulsing mode, but instead it shifts from spontaneous, unsynchronized pulses to high frequency, highly regulated and synchronized pulses.

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