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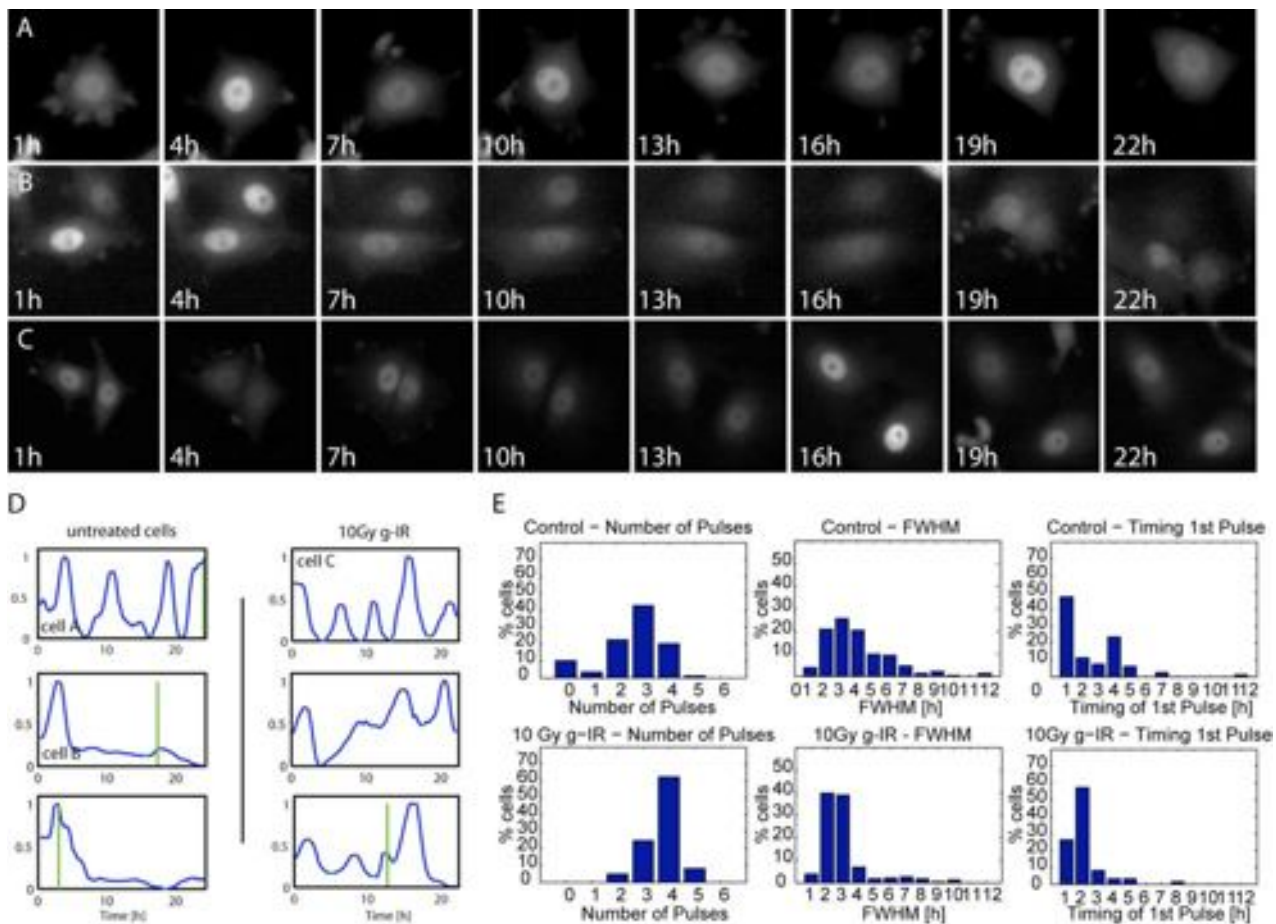
## Abstract

The tumor suppressor p53 is a central player in the signaling network guarding the integrity of our genome. The p53 network, one of the best studied biological systems, 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 most prominent one mediated by the ubiquitin ligase Mdm2, counteract p53 activation by inducing its degradation [2]. The architecture of these feedback loops shapes the dynamic response of the pathway. Ionizing radiation, for instance, leads to a series of undamped pulses of p53 protein [3, 4]. It is, however, unknown what the dynamic behavior of p53 is in the absence of exogenous stimuli: Is the refined network able to maintain a low, steady level of p53, or can we observe spontaneous pulses of p53?

To address these questions, we refined our single cell approach [4]: we fused Venus, a fast maturing fluorescent protein to p53, transfected the fusion protein to MCF7 cells, and selected a highly efficient clonal cell line. This clone allows us to detect and measure p53 basal levels in the absence of external stimuli. We then used fluorescence microscopy to follow p53 levels in irradiated and nonirradiated single cells over time. As previously observed, ionizing radiation triggers periodic uniform pulses of p53 protein (Figure 1C). But interestingly, we observed increases in p53 levels even in untreated cells (Figure 1A,B). To ensure an unbiased analysis of p53 dynamics, we used a pulse detection algorithm to identify the p53 pulses in single cell trajectories and evaluated the characteristics of these pulses, including their duration and timing. Our analysis showed that while irradiation triggers an immediate series of pulses in all cells, the pulses in unstressed cells are non-synchronous and appear to be random. In addition, the duration of p53 pulses in unstressed cells is less uniform (Figure 1D-E). To determine if these complex dynamics are limited only to MCF7 cells, we used immuno-fluorescence studies on various cancer cell lines and immortalized primary cells, and found that endogenous p53 shows similar dynamic behavior.

But how are these pulses generated? Are they the consequence of the activation of upstream kinases, for example by spontaneous DNA damage or replicative stress, or are they caused by stochastic fluctuation in the core feedback loop between p53 and Mdm2? Another interesting question is whether p53 accumulation in unstimulated cells leads to activation of its target genes, or if the signaling network is able to filter spontaneous p53 pulses. I will discuss how we addressed these questions by using small molecule inhibitors of upstream kinases or siRNA against key players in the network.

Taken together, our results show that the p53 network is more dynamic than previously recognized and frequently activated even in unstressed cells. This highlights the importance of time resolved single cells studies, as most of the observed phenomena are masked by the averaging effect of population studies, or are lost when only single time points are evaluated.



**Fig. 1** A-C) Examples of time lapse microscopy experiments using MCF7-p53Venus cells. Cells in A) and B) were not treated, cells in C) were irradiated with 10Gy  $\gamma$ -IR. D) Sample trajectories of cells left untreated or irradiated with 10Gy  $\gamma$ -IR. The trajectories of the examples shown above are indicated. Green vertical bars indicate cell divisions, red and green dots indicate peaks and troughs of p53 pulses as determined by a pulse detection algorithm. E) Histograms showing the distributions of pulse number, duration and the timing of the first pulse in untreated cells in comparison to irradiated cells. FWHM – full-width at half maximum.

## References

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