Functional Roles of p53 Dynamics in Regulating Target Gene Expression

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***Short Abstract* — The tumor suppressor p53 is among a growing list of transcription factors that show complex pulsatile dynamics in response to stimuli. While p53 pulses have been shown to impact cell fate decisions, the mechanisms by which this regulation occurs remain poorly understood. Here, we describe our recent computational and experimental efforts in which we identify specific functions of p53 dynamics in the regulation of its numerous target genes. Our findings point to general principles that will likely be important for understanding a variety of pulsatile systems.**

***Keywords* — dynamical systems, pulsatile transcription factors, p53, live-cell imaging, single-cell transcriptional profiling**

I. Purpose

CELLS use complex signaling pathways to detect environmental stimuli and execute appropriate responses. As methods for quantifying intracellular signaling have improved, several signaling pathways have been found to transmit information using signals that pulse in time [1, 2]. The transcription factor p53 is a key stress-response regulator that exhibits pulsatile dynamics [3, 4]. In response to DNA double-strand breaks, p53 levels in the nucleus increase in pulses with a fixed amplitude, duration, and period; the mean number of pulses increases with DNA damage [3].

p53 regulates the expression of over 100 target genes involved in a range of cellular stress responses including apoptosis, cell cycle arrest, senescence, DNA repair, and changes in metabolism [5]. p53 pulsing directly impacts p53 function: altering p53 dynamics by pharmacologically inhibiting p53 degradation changes patterns of target gene expression and cell fate [6]. While p53 pulsing serves an important signaling function, it is less clear what it accomplishes mechanistically.

Here we will describe our recent efforts to determine the impact of p53 pulsing on the dynamics and coordination of target gene expression.

II. Results

We used a combination of experimental approaches, both at the population level and at the single-cell level, informed by computational and mathematical modeling efforts, to identify functional consequences of p53 dynamics on target gene expression.

A. p53 pulses diversify target gene dynamics

Using quantitative PCR, we measured the expression of the majority of p53 target genes in response to DNA damage. From these measurements, we determined several distinct classes of target gene dynamics, including pulsatile, rising, and step-like dynamics. Using mathematical modeling, we identified mRNA half-life as an important parameter in determining expression dynamics.

B. Subnetwork architecture in the p53 network

Using single-cell transcriptional profiling at key time points following damage, we identified specific subnetworks of co-regulated p53 target genes. The majority of target genes composing the major subnetwork were genes with pulsatile expression dynamics.

C. Tools to control p53 dynamics

We used pharmacological and novel synthetic biological approaches to control the dynamics and localization of p53 in individual single cells. Using these tools, we are identifying the roles of specific characteristics (amplitude, duration, and period) of p53 dynamics on the activation of p53 target genes.

III. Conclusion

Our results give new insight into the function of a growing number of pulsatile signaling pathways and may inform chemotherapeutic strategies based on manipulation of p53 dynamics.

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