A systems-driven experimental approach reveals the complex regulatory distribution of p53 by circadian factors

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Unlike single-cell organisms with self-contained timekeeping systems, multicellular organisms partition their oscillators among different cell types and depend on more complex molecular networks to sense signals and coordinate effective responses. We found that the core circadian clock protein Period 2 (Per2) directly interacts with the checkpoint regulatory component p53, promoting its stabilization and controlling p53 transcriptional activity. Remarkably, circadian phases of Per2 and p53 are anti-phase in the cytoplasm and in-phase in the nucleus, posing new questions about the extent to which Per2 association modulates p53 distribution. Therefore, we focused our efforts on investigating what simulated conditions better relate to the experimental data using mathematical models. Specifically, the model predicted that the phase of the Per2:p53 interaction strongly depends on the binding mechanisms between Per2 and p53 mediated by ubiquitin, as determined by evolving the interaction types between Per2 and p53 in the model during the fitting process. As a result, the ubiquitilation state of p53 impacts Per2 binding and subcellular distribution. All predictions were confirmed experimentally.

Keywords **– circadian rhythms, p53, Period 2 (Per2), reverse engineering technique, protein shuttling.**

I. INTRODUCTION

A KEY ASPECT of cell homeostasis in multicellular systems involves synchronizing cells to changes in environmental conditions, which results in coordinated responses that influence cell proliferation and death. Our previous findings indicate that the circadian sensor factor Per2 directly acts at the p53 node of the checkpoint response influencing various levels of regulation that impact cellular metabolism and bioenergetics ultimately supporting growth and proliferation. In this study, we unveil the time-dependent regulatory mechanisms that modulate p53's oscillatory behavior, stability, and cellular distribution through its association to Per2 using, initially, mathematical models. Predictions were validated and expanded by experimental data.

Acknowledgements: This work was funded by NSF DMS-0931642 to the Mathematical Biosciences Institute (J.K.K.) and NSF CAREER Award to C.V.F. (MCB-0844491)

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II. RESULTS

Our recent study finds that Per2 forms a trimeric complex with p53 and its negative oncogenic regulator Mdm2 [1]. In unstressed cells, this association leads to increased p53 stability through blocking Mdm2-dependent ubiquitination and transcription of p53 target genes. Despite these findings, when levels of these proteins were monitored in total extracts, circadian phases of p53 and Per2 were anti-phase. Subcellular fractionation provided a more comprehensive picture of their distribution and revealed that p53 and Per2 were anti-phase in the cytoplasm but in-phase in the nuclear fraction. To investigate the mechanisms underlying these unexpected phase relationships, we initially used mathematical modeling, where the interaction types between p53 and Per2 stochastically evolved during the parameter fitting process. Using this approach and timecourse data, we inferred that *i*) the half-life of p53 in the nucleus should be greater than that of the cytosolic-localized protein and that *ii*) p53 nuclear entry should be mediated by Per2. These predictions were confirmed experimentally. Overall, our data supports a model in which time-dependent phase shift accumulation of Per2 and p53 proteins results from a delay in post-translational modification events that take place in separate cellular compartments.

III. CONCLUSION

Our ongoing hypothesis is that Per2 helps to maintain basal levels of p53 in unstressed cells to "prime" the signaling pathway to rapidly respond to a stress condition (*i.e.*, metabolic, genotoxic). Our new data expand the current model to include regulation of their interaction by post-translational mechanisms and cellular compartmentalization as evidenced by modeling, and was proven experimentally. In fact, while previous studies have focused on using oscillating timecourse data to infer the presence of interactions among components of biochemical networks [2], our mathematical approach allows the use of timecourse data to further predict additional signature-types needed for molecular interactions to occur in specific cellular compartments.

REFERENCES

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