Multi-scale Robustness analysis revealing novel cell cycle regulators in extreme conditions

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***Short Abstract* — Multi-scale robustness analysis can exploit the predictive power of *in silico* biological models. We present an integrative systems-level approach for seeking novel regulators that contribute to the robustness of a regulatory network. Based on a hybrid model of the *Caulobacter* cell division cycle, we applied various analytical tools to the *in silico* model in order to identify vulnerable operating points in both space and time. Replicating these conditions *in vivo* has led to the experimental discovery of novel regulatory functions that balance the levels of other master cell cycle regulators to rescue the cell cycle in extreme growth conditions.**

***Keywords* — robustness, cell cycle, *Caulobacter*, asymmetric cell division**

I. Purpose

Cell-division cycle is a fundamental process, which controls many aspects of development. We study *Caulobacter crescentus*, a popular model for understanding bacterial asymmetric division, which shares many common features with that of eukaryotic stem cells. In addition to ODE based models published by other groups, we have shown that a hybrid *in silico* model was able to simulate the entire cell cycle by accommodating the complexity and incompleteness of our understanding [1-2]. A great number of discoveries have recently been made on the spatial mechanisms that are critical to the asymmetric cell division [3-4], making it more challenging to understand the integrated time-space feedback controls formed in the cell cycle regulatory network. We are also starting to discover subtle, non-essential regulatory motifs that do not simply turn on or off a cell function, but make cell cycle robustness under various.

II. Materials and Methods

We conducted a multi-scale robustness analysis on an updated *Caulobacter* cell cycle model to look for novel regulatory functions. We first performed a parameter sensitivity analysis to identify the critical parameters to which the *in silico* cell cycle model was sensitive. A following Monte Carlo simulation gave us a measure of robustness in terms of how often the cell cycle would fail when parameters deviate from their nominal values. Next, we performed a more rigorous analysis using symbolic modeling checking (SMV), which is a formal verification tool developed in engineering for checking signal timing [5]. SMV essentially varies the relative timing of all the cell cycle events and searches the entire state space in order to captures "logic errors", or wrong discrete decisions made by the cell cycle control circuits. A more detailed stochastic analysis using the SSA solver was then performed on selected pathways to yield more insights. The multi-scale robustness analysis provided a list of specific clues for finding regulators that were missing in the *in silico* model.

III. Results

The robustness analysis generated a list of conditions under which the cell cycle would fail without the help of additional regulators. However, experimental evidence showed that cells could still divide under these predicted conditions, hence proving the existence of such novel regulators missing in the model that are capable of rescuing the cell cycle.

We discovered a new regulator, BmrA, which forms multiple nested feedbacks with the master cell cycle regulator CtrA, DnaA, and CcrM. The regulatory role of BmrA was not detectable under normal cell division rates. However, under both fast and slow growth conditions, BmrA dramatically reduces the failure rate of the cell cycle by balancing the relative levels between the master regulators. We also found extra methylation mechanisms and promoters that work synergistically to improve the robustness of the *Caulobacter* cell cycle.

IV. Conclusion

Multi-scale robustness analysis allows computational models to predict new regulatory functions that are not obvious from simulations. It also provides specific clues that enable experimentalists to discover the exact mechanisms behind them.

References

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