Hybrid modeling and robustness analysis on *ter* cell cycle regulation

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Intro We have used a hybrid model to analyze the cell cycle control system of the bacterium *Caulobacter crescentus.* We analyzed the robustness of the cell cycle regulatory network using a validation methodology from engineering, which led to the discovery of previously unrecognized robustness mechanisms in this organism.

Cell cycle regulation *Caulobacter* is a model organism for studying bacterial cell cycle regulation and asymmetrical division[1]. The *Caulobacter* cell cycle progresses through three distinct stages: swarmer, stalked, and pre-division (Fig. 1A), during which the cell sheds its flagellum, grows a stalk, replicates its DNA, and divides. All these cell functions are controlled by a regulatory network (Fig. 1B). A genetic circuit comprised of four master regulator proteins, DnaA, GcrA, CtrA, and CcrM, forms the core engine of the regulatory network[2]. At the swarmer-to-stalked transition, the depletion of DNA replication inhibitor CtrA signaled by the CckA phospho-pathway enables chromosome replication. The replication fork passes through the *dnaA*, *ctrA*, and *ccrM* genes in succession, leading to hemi-methylation of their promoters to alter their transcriptional activity. This epigenetic mechanism involving methylation provides a way of synchronizing the core cell cycle engine with the progression of DNA replication. The tubulin-like FtsZ-ring contracts and compartmentalizes the cell, physically blocking the nascent stalked daughter cell from the CckA kinase localized at the swarmer pole, which triggers depletion of CtrA~P and enables initiation of DNA replication in the stalked daughter cell.

Hybrid modeling Activity of the master regulator proteins is closely coupled with cell cycle progression through feedback signals indicating the rates of progression of cell functions such as DNA replication (Fig. 1C). Hence it is necessary to model the cell functions to ensure correct timing for the regulatory network even though they are hard to model mechanistically using continuous ODEs due to their complexity. In our novel hybrid modeling approach, the cell functions, modeled empirically as discrete event driven logic, and the regulatory network, modeled as continuous ODEs, are integrated in a hybrid simulation to mirror the *in-vivo* feedback control (Fig. 1D). The resulting model has been invaluable in investigating the roles of various cell regulated factors. The levels of regulatory proteins and mutant phenotypes are predicted accurately by the simulation.

Robustness Stochastic effects as well as environmental and genetic perturbations *in vivo* could affect parameter values in the model, changing the timing of the cell cycle regulation (Fig. 2A)[3]. Interestingly, sensitivity analysis of the model reveals that the genetic circuit architecture produces relative insensitivity of successful cell cycle operation to large parameter variations[4-8].

For analysis of the robustness of the cell cycle control, we used an engineering circuit timing verification tool, NuSMV[9], to exhaustively identify the extreme conditions under which the cell cycle would fail to complete. Without assuming any particular values for the model parameters,

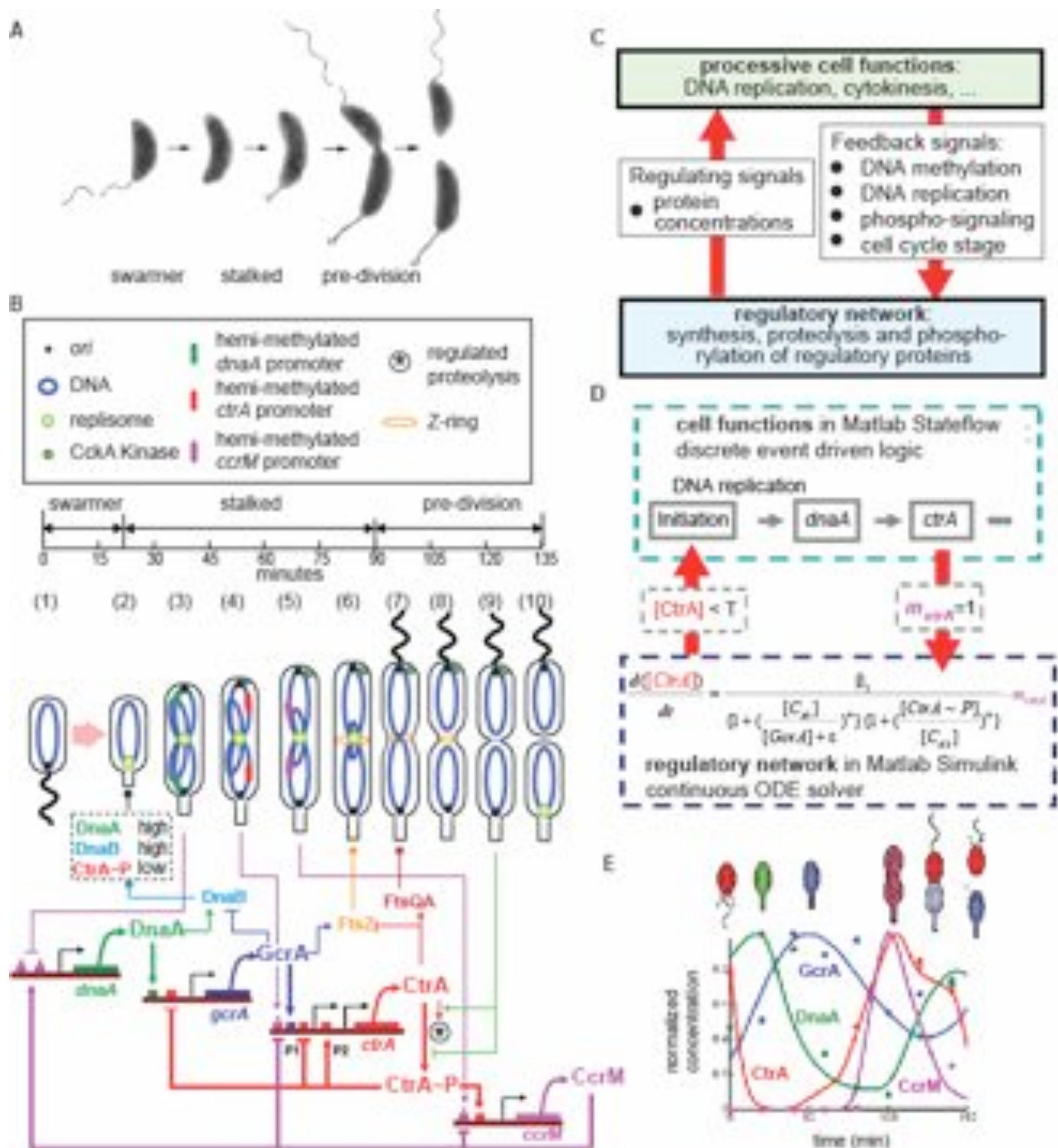

Figure 1. Hybrid model of *Caulobacter* cell cycle regulation. **(A)** The asymmetric *Caulobacter* cell cycle division produces a swarmer daughter cell and a stalked daughter cell**. (B)** A regulatory network regulates the cell functions. A cascade of four master regulator proteins: DnaA, GcrA, CtrA, and CcrM, forms the core engine of the network. Ten unique states of the dividing cell, (1) ~ (10), are shown with reference to time. The depletion of CtrA~P causes the stalked cell to initiates DNA replication (2) and GcrA synthesis. The replication fork passes and hemi-methylates the *dnaA* (3), *ctrA* (4) and *ccrM* (5) promoters in succession, altering their transcriptional activity (purple arrows). The ensuing synthesis of FtsZ (6) and FtsQA (7) are required for the Z-ring formation and contraction. The inner membrane compartmentalization (9) blocks the CckA kinase at the swarmer pole from the stalked daughter cell, thus degrading and unphosphorylating CtrA~P (green arrows) in the stalked daughter cell to enable DNA replication (10). **(C)** The regulatory network and the cell functions form feedback controls. **(D)** A hybrid *in-silico* model architecture mimicking the *in-vivo* cell cycle control shown in (C). Integrated in Matlab, the discrete cell function models and the continuous regulator models interact in *real time*. **(E)** Results of the wild type simulation. The color coded curves are simulated protein levels. The color coded dots are corresponding protein level easurements from western blots. The cell symbols show the corresponding cell states as the simulated cell progresses in time.

the tool traces every trajectory in the state space to find cases that fail to initiate and complete all the cell functions (Fig. 2BCDE). This analysis found few cycle-breaking scenarios, attesting the robustness of the regulatory network. Further experiments showed that previously unrecognized mechanisms make cell cycle extra robust to these scenarios (Fig. 2F). The transition between cell division and growth arrest by starvation is also shown to be regulated robustly.

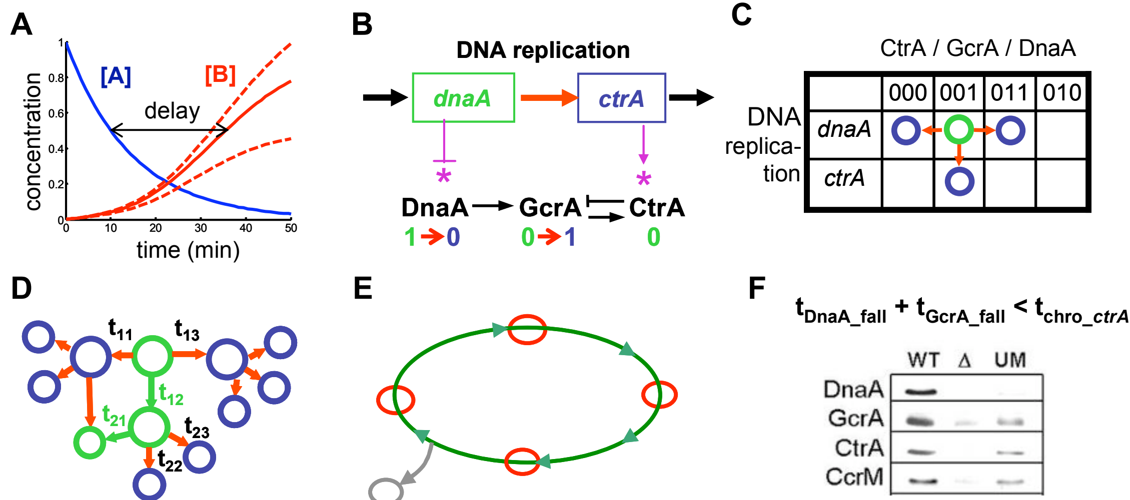

Figure 2. Robustness analysis of the *Caulobacter* cell cycle model. **(A)** Parameter variation affects protein regulator timing. E.g., the delay between the depletion of repressor A (blue curve) and the accumulation of protein B (red solid curve) changes when the half-life of protein B is varied by 30% (red dotted curves). **(B)** An instance when the DNA replication state and the regulator concentration levels are all changing simultaneously. The outcome depends on which transition completes first. Green denotes the current states; blue denotes the next states; red denotes the simultaneous transitions. **(C)** A state transition table showing the possible transitions. **(D)** Each trajectory in the state space corresponds to a specific timing scenario. The one shown in green is when $t_{12}<t_{11}$, $t_{12}<t_{13}$, $t_{21}<t_{22}$, $t_{21}<t_{23}$. All trajectories (timing scenarios) have to be tested by the robustness analysis. **(E)** A trajectory (green) matches a successful cell cycle when it passes through all the key cell-cycle states (red circles) to initiate and complete the necessary cell functions. The failed trajectories, usually stuck in some dead-end states (gray circle), are reported along with their timing condition by the tool. **(F)** One of the few cycle-breaking timing scenario reported by the tool, when DnaA and GcrA are depleted ($t_{DnaA_fall} + t_{GcrA_fall}$) before the replication fork reaches the *ctrA* promoter (t_{chro_ctrA}). Corresponding experiments discovered that leakage expression of un-methylated *dnaA* promoter is able to revive cell cycle under this extreme condition (shown in the UM column of the western blots), further proving the robustness of cell cycle regulation.

Conclusion Hybrid modeling with timing validation has proven effective in analysis of the operation and robustness characteristics of the *Caulobacter* cell cycle control system.

1. McAdams, H. H. & Shapiro, L. A bacterial cell-cycle regulatory network operating in time and space. Science 301, 1874-7 (2003).
2. Collier, J., Murray, S. R. & Shapiro, L. DnaA couples DNA replication and the expression of two cell cycle master regulators. Embo J 25, 346-56 (2006).
3. Elowitz, M. B., Levine, A. J., Siggia, E. D. & Swain, P. S. Stochastic gene expression in a single cell. Science 297, 1183-6 (2002).
4. Savageau, M. A. Parameter sensitivity as a criterion for evaluating and comparing the performance of biochemical systems. Nature 229, 542-4 (1971).
5. Morohashi, M. et al. Robustness as a measure of plausibility in models of biochemical networks. J Theor Biol 216, 19-30 (2002).
6. Barkai, N. & Leibler, S. Robustness in simple biochemical networks. Nature 387, 913-7 (1997).
7. Alon, U., Surette, M. G., Barkai, N. & Leibler, S. Robustness in bacterial chemotaxis. Nature 397, 168-71 (1999).
8. von Dassow, G., Meir, E., Munro, E. M. & Odell, G. M. The segment polarity network is a robust developmental module. Nature 406, 188-92 (2000).
9. http://nusmv.irst.itc.it/.