Switch-like negative feedback promotes clock-like mitotic oscillation

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Short Abstract — Xenopus **early embryonic cycles proceed in a clock-like fashion. These cycles are driven by a biochemical oscillator with many molecular components identified. Here we combine theory and experiments to determine what is required for this circuit to operate robustly and precisely. We reduce the system to its essence, a two-component model where Cdk1-cyclin B1 and APC-Cdc20 regulate each other through interlinked positive and negative feedbacks. Theoretical analysis reveals that to obtain robust and precise oscillations, the steady state response of APC to Cdk1 must be significantly ultrasensitive. We confirm this prediction by developing a real-time fluorescence-based assay in *Xenopus* extracts and obtaining the response function experimentally. Our results suggest that switch-like negative feedback may be a useful strategy for converting a positive-feedback-driven bistable switch into an autonomous, clock-like oscillator.**

Keywords **— Activator-repressor topology, nonlinear dynamics, bifurcation analysis, clock-like embryonic cycles.**

I. Introduction

Unlike the somatic cell cycle, which undergoes four consecutive phases maintained by three major checkpoints, the early *Xenopus* embryonic cycle simply oscillates between S- and M-phases every 25-30min, much like a clock [1,2]. Substantial progress has been made in dissecting the regulatory circuit that drives these oscillations. The circuit is centered on the protein kinase Cdk1-cyclin B1, which, when active, drives the embryo into M-phase, and the E3 ubiquitin ligase APC-Cdc20, which tags cyclin B1 for destruction and drives the embryo out of mitosis. Together, the Cdk1/APC circuit forms a negative feedback loop.

The Cdk1/APC circuit also includes three positive feedback loops. Cdk1 activates its positive regulator Cdc25C, and inactivates its negative regulators Wee1A and Myt1. These feedback loops function as a bistable trigger for mitosis [3, 4], a toggle switch that flips between a low-Cdk1 activity interphase state and a high-Cdk1 activity mitotic state. This toggle switch is converted into an oscillator through the agency of the Cdk1/APC negative feedback loop.

II. Results

Here, we examined how the qualitative character of the negative feedback—e.g. the shape of APC's response function—may affect the ability of the Cdk1/APC system to generate robust oscillations. To capture the essence of the system, we used a relatively simple two-ODE model of the interlinked positive and negative feedback loops. Using the methods of nonlinear dynamics, we discovered that only the sigmoidal-shaped response function of APC to Cdk1 allows for limit cycle oscillations while neither linear nor hyperbolic response can. More importantly, this ultrasensitive response plays a key role in keeping robust and precise oscillations over a large parameter space, which may explain how the clock-like behavior is maintained against environmental changes during embryonic cleavages.

To test our model prediction, we developed a real-time fluorescence-based assay in *Xenopus* extract and monitored the degradation rate of the APC substrate, cyclin B1-CFP, as a surrogate for APC-Cdc20 activity. This allowed us to quantitatively characterize the response function of APC-Cdc20 to its kinase activator Cdk1-cyclin B1, which indeed turned out to be significantly ultrasensitive.

III. Conclusion

In summary, we have shown through modeling that highly ultrasensitive response functions in an oscillator's negative feedback loop promote robust, precise oscillations, and have found that for the *Xenopus* embryonic cell cycle, the negative feedback from Cdk1-cyclin B1 through APC-Cdc20 is, in fact, highly ultrasensitive. The mechanism underpinning this ultrasensitivity remains to be determined. Nevertheless, given how common interlinked positive and negative feedback loops are in biological oscillators [5] as well as some synthetic oscillatory networks [6], these findings suggest that ultrasensitive negative feedback may prove to be a recurring theme in biological clocks.

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