

A synthetic oscillator couples to the cell division cycle in budding yeast

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Short Abstract — Living systems have evolved different natural oscillations, such as the cell division cycle, metabolic rhythms and circadian clocks. These oscillators play important biological roles in the survival and the function of cells. These different oscillations often co-exist in the same cell with variable periods, yet are expected to affect and synchronize with each other even with weak coupling. Here we developed a synthetic oscillator in budding yeast using negative feedback loop based on protein sequestration, a key mechanism of natural oscillators. We show that our synthetic oscillator is autonomous but strongly coupled to the cell cycle in proliferating cells.

Keywords — Synthetic oscillator, budding yeast, protein sequestration, cell cycle coupling and mixed feedback loop.

I. INTRODUCTION

Living systems have different oscillations such as the cell division cycle, metabolic rhythms and circadian clocks [1,2]. Organisms have evolved several genetic oscillators, many of which use protein sequestration as a key mechanism to generate negative feedback. For example, a common architecture is the mixed feedback loop (MFL) [3], which is a two-gene circuit that consists of a constitutive activator and an inhibitor driven by activator homo-dimer binding. The negative feedback occurs when the activator produces high levels of inhibitor that eventually sequester the activator into an inactive hetero-dimer complex. To better understand the design principles of sequestration-based oscillators, we have built a synthetic MFL circuit in budding yeast.

Strikingly, our synthetic oscillator was strongly locked to the budding yeast cell cycle. We verified that the synthetic oscillator was autonomous by blocking the yeast cell cycle with nocodazole. The blocked MFL circuit exhibited autonomous oscillatory dynamics with a period similar to wild-type cell cycle. We could also modulate the MFL oscillator period by changing the inhibitor degradation rate

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via a tunable degron [4]. However, the autonomous period in blocked cells did not change much relative to the cell cycle period, which explained why the MFL continued to exhibit 1:1 locking with cell cycle in non-blocked cells over a range of degradation rates. These results are different from those with bacterial synthetic oscillators [5,6], which did not couple to the underlying cell cycle. We are currently testing whether this arises from fundamental differences in cell cycle biology and/or synthetic oscillator design.

II. CONCLUSION & DISCUSSION

We have constructed a genetic oscillator in budding yeast that uses the mechanism and the topology frequently found in natural oscillators, i.e. mixed feedback loop (MFL) based on the protein sequestration. Our synthetic oscillator was capable of exhibiting autonomous oscillations with varying periods via a tunable degron of the inhibitor in cells with a blocked cell cycle. However, the MFL would always couple to the cell division cycle in proliferating cells.

Our work shows that yeast cell cycle can have a strong coupling with endogenous oscillators. That, in turn, raises questions as to whether cell cycle coupling is a universal feature across all eukaryote organisms and whether the cell cycle can impact the evolution of other natural oscillators and vice versa.

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