

# In vitro cell cycle oscillations exhibit a robust and hysteretic response to changes in cytoplasmic density

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**Abstract**—The cytoplasm, where most cellular reactions occur, has a variable density. However, we lack an understanding of how density variations affect cellular functions because controlling density experimentally is challenging. Here, we systematically modulate the density of an in vitro cytoplasm using microfluidics, and analyze how the cell cycle responds. We found that mitotic cycles maintain their function between 0.2× and 1.2× of the natural density. Higher densities arrest cell cycles, and dilution recovers oscillations. However, the density at which cycles reappear is lower than the natural density. This behavior suggests a history-dependent mechanism called hysteresis, common in physics, chemistry, and engineering. Our approach paves the way for studying the responses of other processes to density changes.

## I. INTRODUCTION

**R**obustness, or the ability to maintain function despite environmental perturbations, appears ubiquitous across many areas of biology. Processes as diverse as bacterial chemotaxis, osmoregulation, circadian rhythms, and development have been found insensitive to certain conditional changes over a wide range of magnitudes. Rather than requiring the precise tuning of parameters, studies have attributed the stability of their functioning to a robust property of their underlying biochemical networks.

At the cellular level, biochemical reactions take place in the cytoplasm, a highly complex and dynamic environment. Activities including synthesis, degradation, osmosis, endocytosis, and exocytosis may all perturb the eukaryotic cytoplasm. It is crucial to understand how cytoplasmic properties vary and how internal processes respond to these fluctuations.

Cells control the properties of the cytoplasm to ensure proper functioning of biochemical processes. Recent studies showed that cytoplasmic density varies in both physiological and pathological states of cells undergoing growth, division, differentiation, apoptosis, senescence, and metabolic starvation. Little is known about how cellular processes cope with these cytoplasmic variations. Here, we study how a cell

cycle oscillator comprising cyclin-dependent kinase (Cdk1) responds to changes in cytoplasmic density.

We systematically dilute and concentrate cycling *Xenopus* egg extracts in cell-like microfluidic droplets. We used a programmable pressure-driven control of liquid flow in a microfluidic device to generate droplets encapsulating extracts with different dilution factors. We then measured the period and persistence of oscillations in these droplets using a cyclin-dependent kinase (Cdk1) activity fluorescence resonance energy transfer (FRET) sensor.

## II. RESULTS

We found that the cell cycle maintains robust oscillations over a wide range of deviations from the endogenous density: as low as 0.2× to more than 1.22× relative cytoplasmic density (RCD). A further dilution or concentration from these values arrested the system in a low or high steady state of Cdk1 activity, respectively. Interestingly, diluting an arrested cytoplasm recovers oscillations at lower than 1× RCD. Thus, the cell cycle switches reversibly between oscillatory and stable steady states at distinct thresholds depending on the direction of tuning, forming a hysteresis loop.

We developed a mathematical model to investigate the role of cytoplasmic density in the oscillatory behavior of the cell-cycle network. Our model recapitulates the robustness observed in the system and predicts that the Cdk1/Wee1/Cdc25 positive feedback loops do not contribute to the observed robustness. Experiments support this prediction and further characterize the response of the system to perturbations.

The techniques described here can be applied to study how cytoplasmic density affects other cellular processes.

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