Nuclear-cytoplasmic compartmentalization of cyclin B1-Cdk1 promotes robust timing of mitotic events

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***Short Abstract* — Although the cyclin-dependent kinase 1 (Cdk1) oscillator has been well-characterized in homogenized cytosolic extracts of *Xenopus*, how the nucleus contributes to the accuracy of mitosis remains unclear. Here, we developed a FRET biosensor and compared the Cdk1 spatiotemporal dynamics in reconstituted synthetic cells with or without nuclei. We found nucleocytoplasmic compartmentalization significantly modulates clock properties. In contrast to the Cdk1-inactive and period-tunable interphase in the cytoplasmic cells, cells with nuclei displayed bi-phasic Cdk1 activation and stable period despite cyclin concentration variations. Moreover, by tracing single-cell phase-plane trajectories, we found Cdk1 and cyclin B1 cycle rigorously out-of-phase, producing wide phase-plane orbits, essential for oscillation robustness.**

***Keywords* — cell cycle, synthetic cells, water-in-oil microemulsion droplets, FRET biosensor, single-cell signaling dynamics, microfluidic device**

I. Purpose

OVER the past decades, studies applying well-mixed cytosolic extracts from *Xenopus laevis* have contributed substantially to understanding how the Cdk1-centered network functions faithfully in driving the cell cycle into and out of mitosis [1-4]. However, many subcellular processes in eukaryotic cells are compartmentalized in the membrane-bound nucleus and organelles, to increase their efficiency and specificity. Notably, recent studies have identified spatial positive feedback that drives abrupt cyclin B1-Cdk1 nuclear translocation upon activation [5,6], raising the question of whether the clock properties revealed in homogenized cytoplasmic extracts still hold in single cells containing nuclei. Additionally, it remains unclear the contribution of compartmentalization in temporal accuracy during mitotic entry and the faithful temporal order of various downstream mitotic events driven by Cdk1.

II. Results

A. Development of Cdk1-EV FRET biosensor

For visualization of cyclin B1-Cdk1 kinase activity in both spatially homogeneous and heterogeneous conditions, we developed a new Cdk1 FRET biosensor than a previously reported Cdk1 FRET biosensor [6,7] in cell-free *Xenopus* extracts. Our Cdk1-EV also has a high specificity to the Cdk1 kinase activity. It enables the first experimental frequency-amplitude measurement and demonstrates that Cdk1 oscillator is frequency-modulated and amplitude-constant in homogenized cytoplasm, as theory predicted [8].

B. Biphasic activation of Cdk1 and temporal ordering of mitotic events

To understand the impact of cellular compartmentalization, we added demembranated sperm chromatin to induce the self-assembly of nuclei in droplets. In the nucleus-containing droplets, Cdk1 steadily increased from the beginning of interphase, with a roughly linear dependence with time, to a moderate activity before a quick jump to a distinctively higher activity at NEB.

C. Phase plane mapping between Cdk1 and cyclin B1

We simultaneously measured the cyclin B1 expression and Cdk1 spatiotemporal dynamics over time to obtain a complete picture of the Cdk1 activation-deactivation cycle in relation to the cyclin synthesis-degradation cycle and their relationships with the downstream nuclear events.

III. Conclusion

Our development of biosensors and droplet-based synthetic cells provides a critical tool for studying differences between the presence and absence of nuclei. The approach has allowed us to reveal the impact of nucleocytoplasmic compartmentalization. It may also be adaptable to exploring the potential functional advantages of other nucleocytoplasmic segregated processes. This work has been submitted to bioRxiv (in revision) [7].

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References

[1] Murray AW (1991) Cell cycle extracts. *Methods Cell Biol.* **36**, 581–605.
[2] Heald R, McLoughlin M, McKeon F. (1993) Human wee1 maintains mitotic timing by protecting the nucleus from cytoplasmically activated Cdc2 kinase. *Cell*. **74**, 463–474.
[3] Sha W, Moore J, Chen K, Lassaletta AD, Yi C-S, Tyson JJ, et al. (2003) Hysteresis drives cell-cycle transitions in Xenopus laevis egg extracts. *Proc Natl Acad Sci U S A*. **100**, 975–980.
[4] Trunnell NB, Poon AC, Kim SY, Ferrell JE Jr. (2011) Ultrasensitivity in the Regulation of Cdc25C by Cdk1. *Mol Cell*. **41**, 263–274.
[5] Santos SDM, Wollman R, Meyer T, Ferrell JE. (2012) Spatial positive feedback at the onset of mitosis. *Cell*. **149**, 1500–1513.
[6] Gavet O, Pines J. (2010) Progressive activation of CyclinB1-Cdk1 coordinates entry to mitosis. *Dev Cell*. **18**, 533–543.
[7] Maryu G, Yang Q. (2021) Nuclear-cytoplasmic compartmentalization promotes robust timing of mitotic events by cyclin B1-Cdk1. *bioRxiv*.. p. 2021.07.28.454130. doi:10.1101/2021.07.28.454130
[8] Tsai TY-C, Choi YS, Ma W, Pomerening JR, Tang C, Ferrell JE Jr.(2008) Robust, tunable biological oscillations from interlinked positive and negative feedback loops. *Science*.**321**, 126–129.