Single-cell E2F Dynamics Reveal the Control Logic of Cell Cycle Entry

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***Short Abstract* — To explore the molecular events responsible for cell cycle entry, we developed an integrated platform to follow E2F dynamics at the single cell level and in real time. By linking the characteristics of E2F dynamics to phenotypic outputs, we uncover a "threshold-firing" mechanism that underlies the transition process: once the amplitude (*Amp*) of E2F activation crosses a critical threshold (Amp_{th}), cells are irrevocably committed to the cell cycle. Our analyses based on modeling and experimental perturbations further suggest an under-appreciated control logic of this regulation: Myc, rather than G1 Cyclins, is critical for the control of entry decision, by boosting *Amp* above Amp_{th}.**

***Keywords* — cell cycle entry; E2F dynamics.**

I. Introduction

Cell cycle entry is a critical decision, which dictates whether a cell proliferates or not [1]. A body of evidence supports the view that this process is controlled by Myc/Rb/E2F network, in particular by modulating E2F activation [2]. Initial activation of the network is largely thought to occur through the combined actions of Cyclin D and E complexes that gradually phosphorylate Rb, leading to the release of E2F and activation of genes required for cell metabolism and DNA replication [3,4]. However, this generally accepted view is hard to reconcile with the observation that phosphorylation of Rb occurs after the restriction point, suggesting that other events be responsible for the initial activation of the Rb/E2F network [5].

Conventional approaches based on population analysis cannot adequately address this question, in light of extensive heterogeneity in gene expression among cells that can result in diverse phenotypic outputs [6]. Single cell analysis provides the opportunity to follow the dynamics of signaling molecules that reflect how an individual cell encodes and decodes information that result in a particular cellular outcome [7]. In light of the integrative role of E2F within the Myc/Rb/E2F network, we reasoned that measuring single-cell E2F dynamics under different conditions and correlating them with phenotypic outputs would provide mechanistic insights about how cell cycle entry process is determined.

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II. Results Summary

A. Measure E2F dynamics at the single cell level

By performing live cell imaging using an engineered E2F transcriptional reporter cell line, we provided the first glimpse of real-time dynamics of E2F transcription at the single cell level during the mammalian cell cycle and then defined a set of metrics that can quantify these trajectories.

B. Link E2F dynamics to phenotypic outputs

Combining the monitoring of E2F dynamics and the measurements of DNA synthesis/cell division, we uncover that one of the metrics, *Amp*, serves as a highly accurate predictor (up to 96% accuracy) for cell cycle entry after mitogenic stimulation, suggesting a "threshold-firing" mechanism that underlies the transition process.

C. A novel control logic of cell cycle entry

Mathematic modeling and experimental perturbations points to a novel control logic: Inhibition of Cyclin D/E complexes has little or no effect on cell cycle commitment as it fails to prevent *Amp* to rise upon serum stimulation. However, it results in significant variation of the cell cycle length. In contrast, Myc is critical for cell cycle commitment, as its inhibition prevents *Amp* to reach Amp_{th}.

III. Conclusion

Our studies coordinate gene regulation, E2F dynamics and phenotype at the single-cell level, providing mechanistic insights about how cell cycle entry is controlled in individual cells.

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