Quantitatively monitoring the G1stability in single cell

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***Short Abstract* —G1 (START) checkpoint interprets a flood of signals that influence cell division and cell fate. However, our current knowledge about the G1 checkpoint is mostly qualitative. We built a single cell assay to quantitatively monitor the G1 stability and the kinetic of G1/S transition in yeast cell cycle. By using fluorescent microscopy, the kinetic process of G1/S transition can be visualized in single cell in real time. Coupled with computational modeling, this quantitative assay will be used to elucidate various and compounded factors leading to the G1 checkpoint arrest or bypass.**

***Keywords* — Cell cycle, G1/S transition, G1 stability, Cln3, Sic1, Budding yeast**

EXTENDED ABSTRACT

Cell cycle is the essential mechanism of by which all living things reproduce[1]. Decades of work in genetics and biochemistry as well as the more recent work in genomics and proteomics have elucidated many major players during the cell cycle process. Multiple checkpoints were considered to play a very important role in cell cycle regulation [2, 3]. This checkpoint design ensures a robust and orderly cell cycle progression against genetic perturbations, cellular fluctuations, and other uncertainties. Abnormal behaviors of the system as results of perturbations are often associated with unwanted checkpoint bypass or arrest. In particular, the G1 (START) checkpoint interprets a flood of signals that influence cell division and cell fate, and the instability of G1 can leads to cancer [4]. However, our current knowledge about the cell cycle control is mostly qualitative and "linear".

To quantitatively understand the stability of G1 checkpoint, we built a single cell assay to monitor the G1/S transition and its kinetics in budding yeast cell cycle. The cell cycle regulation in budding yeast is one of the best studied biological systems. In budding yeast, the signal to G1/S transition is provided by Cln3 (and to some extent also by Bck2)[5,6].Cln3 (Bck2) integrates information of the environment (nutrient conditions) and the cell itself (cell size) and triggers the G1/S transition when its nuclear concentration exceeds a threshold. In the Δcln3Δbck2 strain, the cell arrests at the START checkpoint [7]. Since we are interested in the checkpoint stability itself, we decoupled the endogenous signaling to the checkpoint from the checkpoint state by building our assay in the Δcln3Δbck2 background. We then used an inducible and quantifiable exogenous signal to trigger the G1/S transition. Two quantitative markers were used to monitor the system: (1) GFP fused with the exogenous signal to monitor the amount of the signal, and (2) an S-phase marker to monitor the G1/S transition and its kinetics. The minimum amount of the signal needed to trigger the G1/S transition would be a measure of the G1 stability. For the signal part, we employed a 2GFP fused Cln3. For the S-phase marker, we constructed a SIC1 marker. Sic1 is a Clb/CDK inhibitor whose rapid degradation in the late G1 correlates well with the onset of the S phase [8]. In order to avoid influence the cell cycle process, we constructed a reporter with the regulatory domain of SIC1 fused with the Cherry-FP gene (the CDK binding domain of SIC1 is deleted in the construct), which has the same nuclear localization and the same degradation kinetics with the endogenous Sic1. This assay system will be crossed with the yeast deletion library to obtain single deletion strains. We believe that, by closely coupling with computational modeling, this quantitative assay can help us to elucidate various and compounded factors leading to the G1 checkpoint arrest or bypass.

A quantitative understanding of the checkpoint stability and instability would represent a significant step forward in our understanding of the cell cycle regulation and might provide clues to the mechanisms and cure of cancer in which the checkpoint instability is a hallmark.

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