Quantitative Measurement for the START Threshold in Budding Yeast Cell Cycle

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***Short Abstract* — We set up a system to measure the threshold of START transition quantitatively and in single cell level. An inducible promoter controlled and fluorescent protein tagged Cln3 mutant was used as the initial trigger of the transition. While the fluorescent protein tagged Whi5 was used to report the timing of the transition. Measuring the threshold of the transition under different growth conditions will facilitate us understanding the basic rules of the coordination between the cell cycle, mass growth rate and cell size at homeostasis.**

***Keywords* — threshold of START, positive feedback loop, yeast cell cycle, nutrient conditions, optimized cell size**

I. Background and Questions

THE checkpoint in late G1 phase is named as START in budding yeast. During G1 phase, the cyclin Cln3 integrates all the external signals and functions as the initial trigger of the cell cycle. Whi5-SBF-Cln1/2 forms a positive feedback loop, makes the transition of START switch-like and irreversible [1, 2 cross irreversible]. In cln3 deletion cell, SBF is activated by a bypass trigger Bck2. When both cln3 and bck2 are deleted, the cell cycle is arrested in G1 phase. Over-expressing Cln3 accelerates the cell cycle entry and results in smaller cell size. When the concentration of Cln3 is reduced to modest level, the cell cycle entry is much delayed, and cells pass the START at larger cell size [3]. Why passing START is time dependent or cell size dependent when the concentration of Cln3 is not sufficient? Mainly, there are three hypotheses. 1) The effect or product of Cln3's activity accumulates over time; the threshold of the START transition is the integration effect of Cln3 instead of the steady state concentration of Cln3. 2) The concentration of Cln3 increases with cell size increasing. Once it reaches the threshold of the START transition, the cell will enter into cell cycle. 3) The threshold of START transition changes with time or cell size. The threshold is lowered when cell wait longer time in G1 phase, or grows to larger cell size. Once the concentration of Cln3 exceeds the threshold, the cell will pass START.

Cell division and growth rate is coupled in START. Cell size at homeostasis is determined by both factors. When cells are switched from rich nutrient medium to poor nutrient medium, the concentration of Cln3 is down regulated, and the threshold of START is reset to lower level as well [4 mike review]. Cells wait longer time in G1 phase and bud at smaller cell size. Cell division and mass growth are two ends of one lever. Is the balanced point optimized in different nutrient conditions? The answer is not clear yet.

II. Quantitative Measurement for the START Threshold

To answer the questions above, we set up a system to measure the threshold of START transition quantitatively. The system was constructed in a cln3 and bck2 double deletion background cell. A mutant Cln3R108A was used as the signal instead of the wild type Cln3, to reduce the binding affinity with CDK1 and increase the half life time [5]. Three tandem fluorescent proteins Venus were fused to the N-terminal of Cln3 to make it visible in single cell under microscope. The protein was controlled by a synthetic inducible promoter GlacSpr, so that it can be titrated by adding different concentration of IPTG. A fluorescent protein mCherry was fused to the C-terminal of the endogenous Whi5 to report the timing of START transition. A microfluidic device was designed to make the cells grow into a single layer and change the inducer concentration and nutrient condition rapidly. Both fluorescent signals of Cln3 and Whi5 were recorded by time lapse microscope. An ODE model was built to fit and explain the experimental data. Measuring the threshold of the START transition quantitatively under different conditions will facilitate us getting insights of the coordination between cell cycle, growth rate and cell size.

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