Timely, decisive switch-like S phase entry is ensured by multisite phosphorylation combined with a double negative feedback loop

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***Short Abstract* — To prevent cells from chromosome instability and thus retain genomic integrity, a proper proteolytic degradation of the S-phase cyclin (Clb5/6)-Cdk stoichiometric inhibitor Sic1 is essential. Cln1/2-Cdk and the requirement for multisite phosphorylation are thought to be responsible for Sic1's switch-like destruction. Here, by using time-lapse fluorescence microscopy, we quantified the initiation timing and the speed of Sic1 degradation separately in single cell. We showed that both the initiation timing and the sharpness of Sic1 degradation are essential. Furthermore, we demonstrated both through computational models and experimentally, that while the multisite phosphorylation is responsible for the robust initiation timing, the double-negative feedback loop between Clb5/6-Cdk and Sic1 is crucial to the robust rapid destruction of Sic1 against genetic and environmental perturbations. In addition, computation results suggested the apparent "redundancy" of Sic1 being a substrate of both the Cln-Cdk and the Clb-Cdk may contribute to the coordination between Start and the S-phase entry.**

***Keywords* — multisite phosphorylation, G1/S transition, positive feedback loop, Timing.**

I. Purpose

THE eukaryotic cell cycle consists of a series of distinct events coordinated by a network of regulatory proteins[1]. In the budding yeast *Saccharomyces cerevisiae*, the commitment to cell cycle is initiated by the G1 cyclin Cln3, which phosphorylates two transcription factors SBF and, MBF, activating the transcription of about 200 G1/S genes including the other two G1 cyclins *CLN1* and *CLN2* [2]. Cln1/2-Cdk in turn promotes their own accumulation, thus forming a positive feedback loop [3]. The transcription of the S cyclins *CLB5* and *CLB6* is also activated at Start together with the other G1/S genes [2]. Unlike Cln1/2-Cdk, Clb5/6-Cdk complexes are rendered inactive throughout G1 phase by the Clb-Cdk inhibitor Sic1 until Sic1 is degraded at the G1/S transition [4]. Strains with either *SIC1* deleted or altered Sic1 degradation kinetics show a significant increase in genomic instability, underlining the importance of Sic1 to prevent precocious activation of Clb-Cdk and for a proper S-phase entry [5]. Multisite phosphorylation by G1 cyclin (Cln)-Cdk is thought to be responsible for Sic1's switch-like destruction [5].

Here, we systematically investigated the influence of various players in the G1/S circuitry on the timing, sharpness, and the variability of Sic1 destruction, and demonstrated a very different picture. By using single cell fluorescence time-lapse microscopy, we were able to quantify the initiation timing and the speed of Sic1 degradation separately, thus assessed their impact on genome stability differentially. We show that both the initiation timing and the sharpness of Sic1 degradation are crucial to the genome stability. By monitoring the dynamics of Sic1 destruction and the nuclear exclusion of Whi5 simultaneously in different Sic1 mutants under different genetic and environmental perturbations, we show that, in contrast to the traditional view, neither the multisite phosphorylation nor the Cln1/2-Cdk is required for the switch-like Sic1 degradation. While the multisite phosphorylation is responsible for the robustness of the Sic1 degradation initiation timing, the robust switch-like degradation is ensured by a double negative feedback loop between Clb5/6-Cdk and Sic1. Our experimental work is supported by computational studies. Furthermore, our computational study suggests the apparent "redundancy" of Sic1 being a substrate of both the Cln-Cdk and the Clb-Cdk may be part of the design, for coordinating the timing between Start and the S-phase entry.

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