Robust switch-like destruction of the CDK inhibitor Sic1 is ensured by a double negative feedback loop

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***Short Abstract* — In eukaryotic cell division cycles, the initiation of DNA replication (S-phase entry) is driven by a sharp rise in the activity of the S-phase cyclin-Cdk. A critical step leading to this G1/S transition in yeast is the rapid proteolytic degradation of the S-phase cyclin (Clb5/6)-Cdk stoichiometric inhibitor Sic1. Multisite phosphorylation by G1 cyclin (Cln)-Cdk is thought to be responsible for Sic1's switch-like destruction. Here, by monitoring Sic1 degradation in real time in individual cells, we show that it is the double-negative feedback loop between Clb5/6-Cdk and Sic1, not Cln-Cdk, that ensures a robust rapid destruction of Sic1 against genetic and environmental perturbations. Our study establishes a new understanding on the control mechanism of G1/S progression and provides insights in the coordination of cell cycle transitions.**

***Keywords* — G1/S transition, positive feedback loop, Robustness, Sic1 destruction.**

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]. However, there are multiple feedback loops in the control circuitry of the G1/S transition. Positive feedback loops are capable of generating all-or-none transitions and are widely implemented in cell fate circuitries [6]. Led by these considerations, we systematically investigated the influence of various players in the G1/S circuitry on the dynamics and the variability of Sic1 destruction.

By monitoring Sic1 destruction in real time in individual cells of different deletion strains, we show that it is the Clb5/6-Cdk, not Cln-Cdk, which ensures a robust rapid Sic1 destruction. In addition, by comparing changes in Sic1 destruction dynamics with and without the double-negative feedback loop, we find that the mutual antagonism between the S cyclin Clb5/6-Cdk and Sic1 filters out noise to ensure a decisive S-phase entry against genetic and environmental perturbations. Furthermore, by employing a simple model of Sic1 destruction, we suggest that a balance on Sic1 phosphorylation is optimal — while high specificity of Clb5/6-Cdk phosphorylation of Sic1 is necessary to ensure a robust sharp rise of Clb5/6-Cdk activity, a certain degree of specificity from Cln1/2-Cdk is desirable to achieve a consistent timing. Thus, the apparent "redundancy" of Sic1 being a substrate of both the Cln-Cdk and the Clb-Cdk may be part of the design for a robust switch coordinating one event with another. This design can achieve robustness in both the timing and the speed of Sic1 destruction, ensuring a coordinated and decisive S-phase entry despite cellular and environmental fluctuations.

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