Multisite phosphorylation is responsible for timing robustness of G1/S transition in yeast

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***Short Abstract* — In budding yeast (*S. Cerevisae*), G1/S transition is triggered by a surge of G1/S cyclin-Cdks, Clb5/6-Cdk. This surge is due to rapid degradation of Sic1, which sequesters Clb5/6-Cdk into an inactive complex. Sic1 has nine consensus Cdk phosphorylation sites, and it degrades rapidly when six of these sites are phosphorylated. Rapid destruction of Sic1 was believed to be a result of multi-site phosphorylation. Our experiments, however, showed that a single phosphorylation site can generate just as sharp a degradation but, variability in initiation timing in this case is much larger. We study the transition using computer simulations to gain insight on how multi-site phosphorylation can decrease the variability in timing of the transition. Preliminary results confirm experiments, suggesting a complex mechanism, in which specificities of kinases involved in the transition differ for each phosphorylation site.**

***Keywords* — Cell cycle, yeast, G1/S transition, Cln, Clb, Sic1, multisite phosphorylation, in silico evolution.**

I. Background

Eukaryotic organisms initiate DNA replication from multiple origins in order to complete the replication of their large genomes quickly. Replication initiation requires a coordinated action between the initiator kinases and the replication machinery to ensure that a large number of origins get fired, and an origin does not get fired more than once per cell cycle [1]. In budding yeast, this is achieved by a switch-like activation of initiator kinases Clb5/6-Cdk: before the initiation, Clb5/6-Cdk is held inactive in a complex by the stoichiometric inhibitor Sic1 [2]. Phosphorylation by the G1-cyclin Cln1/2-Cdk triggers the proteolytic degradation of Sic1. Recent experiments show that free Clb5/6-Cdk can phosphorylate Sic1, which ultimately leads to a rapid destruction of Sic1 [5,6]. This event marks the G1/S transition in yeast cell cycle. Alteration of degradation kinetics or deletion of Sic1 leads to genomic instability [3,5].

Sic1 has nine consensus Cdk phosphorylation sites, and phosphorylation on at least six sites is necessary to trigger its rapid degradation [2]. Earlier studies suggested that the switch-like destruction of Sic1 is due to the multiple phosphorylation events that create an ultrasensitive response [3]. Our experiments show that Sic1 is degraded just as rapidly even with a single optimized phosphorylation site [5]. However, spread of the timing of degradation initiation in the single-phosphosite mutant (1p) is much larger compared to the wildtype (9p). This result suggests that multi-site phosphorylation of Sic1 is reponsible for the timing robustness of G1/S transition in yeast.

II. Purpose and Results

We tested the effect of multisite phosphorylation of Sic1 in timing of G1/S transition using computer simulations. First, we varied the specificities of Cln and Clb kinases, and measured the mean values and variability of timing and sharpness of Clb activation in models with 1p and 9p forms of Sic1. We assumed that each kinase has the same specificity of each phosphosite on the 9p form of Sic1. The results showed no clear advantage of multi-site phosphorylation: 1p performed just as good as 9p.

We then relaxed our assumption, allowing specificities of each kinase to differ for each phosphosite. We implemented an optimization algorithm that resembles evolution by natural selection, in which the specificity configurations that yield sharper transitions and lowest timing variability are selected. Preliminary results confirm the experiments: Multi-site phosphorylation performs better than single site phosphorylation. Simulations also suggest a mechanism, in which phosphorylation sequences of Cln- and Clb-Cdks differ, distinguishing the roles of two kinases.

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