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Sic1 plays a role in timing and oscillatory behaviour of B-type cyclins

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ABSTRACT

Budding yeast cell cycle oscillates between states of low and high cyclin-dependent kinase activity, driven by association of Cdk1 with B-type (Clb) cyclins. Various Cdk1–Clb complexes are activated and inactivated in a fixed, temporally regulated sequence, inducing the behaviour known as "waves of cyclins". The transition from low to high Clb activity is triggered by degradation of Sic1, the inhibitor of Cdk1–Clb complexes, at the entry to S phase. The G_1 phase is characterized by low Clb activity and high Sic1 levels. High Clb activity and Sic1 proteolysis are found from the beginning of the S phase until the end of mitosis. The mechanism regulating the appearance on schedule of Cdk1–Clb complexes is currently unknown. Here, we analyse oscillations of Clbs, focusing on the role of their inhibitor Sic1. We compare mathematical networks differing in interactions that Sic1 may establish with Cdk1–Clb complexes. Our analysis suggests that the wave-like cyclins pattern derives from the binding of Sic1 to all Clb pairs rather than from Clb degradation. These predictions are experimentally validated, showing that Sic1 indeed interacts and coexists in time with Clbs. Intriguingly, a *sic1Δ* strain looses cell cycle-regulated periodicity of Clbs, which is observed in the wild type, whether a *SIC1-0P* strain delays the formation of Clb waves. Our results highlight an additional role for Sic1 in regulating Cdk1–Clb complexes, coordinating their appearance.

1. Introduction

Budding yeast cell cycle is driven by periodic changes in the activity of Cdk1 kinase, regulated by different pools of cyclins that associate with Cdk1 in successive waves (Fig. 1A, reviewed in Futcher, 1996). B-type cyclins Clb1–6 are expressed at different times and appear sequentially in specific cell cycle phases, resulting in a significant divergence of function (Bloom and Cross, 2007; Cross et al., 1999). Clb5,6 rise at the beginning of S phase and function primarily in the control of DNA replication (Schwob and Nasmyth, 1993; Schwob et al., 1994; Spellman et al., 1998). Clb3,4 increase in mid-S phase at about the same time as spindle pole bodies separate and their specific function is still unclear (Fitch et al., 1992; Richardson et al., 1992). Clb1,2 rise as mitotic spindle assembly progresses and are involved in the control of mitotic exit (Deshaies, 1997; Fitch et al., 1992; Spellman et al., 1998). The regulation of active Cdk1–Clb complexes involves a combination of positive feed-forward loops – depending on the regulated transcription of *CLB* genes (Bloom and Cross, 2007; Fitch et al., 1992; Koch and Nasmyth, 1994) – and negative feedback loops – via down-regulation of Clb levels via ubiquitin/26S proteasome pathway (Amon et al., 1994; Hochstrasser, 1995; Irniger et al., 1995; King et al., 1996; Lew and Reed, 1995; Seufert et al., 1995; Tyers and Jorgensen, 2000).

The transcriptional regulation of Clbs is a fine-tuned mechanism (Fig. 1B, reviewed in Bloom and Cross, 2007). *CLB5,6* transcription is promoted by the Mbp1/Swi6 Binding Factor (MBF) (Schwob and Nasmyth, 1993), and interactions of Clb5,6 with Swi4/6 Binding Factor (SBF) and MBF have been reported (Pic-Taylor et al., 2004; Simon et al., 2001). *CLB1,2* transcription is controlled by the Fkh2 forkhead transcription factor during G_2/M phase (Kumar et al., 2000; Reynolds et al., 2003) and both Cdk1–Clb5 and Cdk1–Clb2 interact with, and phosphorylate, Fkh2 to control Clb1,2 accumulation (Hollenhorst et al., 2000; Pic-Taylor et al., 2004; Ubersax et al., 2003; Yeong et al., 2001) (Fig. 1B, arrows C and D, respectively). No information is available so far about the activation of *CLB3,4* transcription. The only study reported to date is a genome-wide location analysis to identify binding sites for transcription factors, which suggested that the Fkh1 forkhead transcription factor binds to Clb4 (Simon et al., 2001). Moreover, Fkh1 binds to the *CLB4* promoter (CCDB database, Alfieri et al., 2007).

The degradation of Clbs by proteolysis is a highly specific mechanism (Fig. 1C), with the consequence that Cdk1 is inactivated. Clb6 is the only B-type cyclin to be directed to degradation by the ubiquitin

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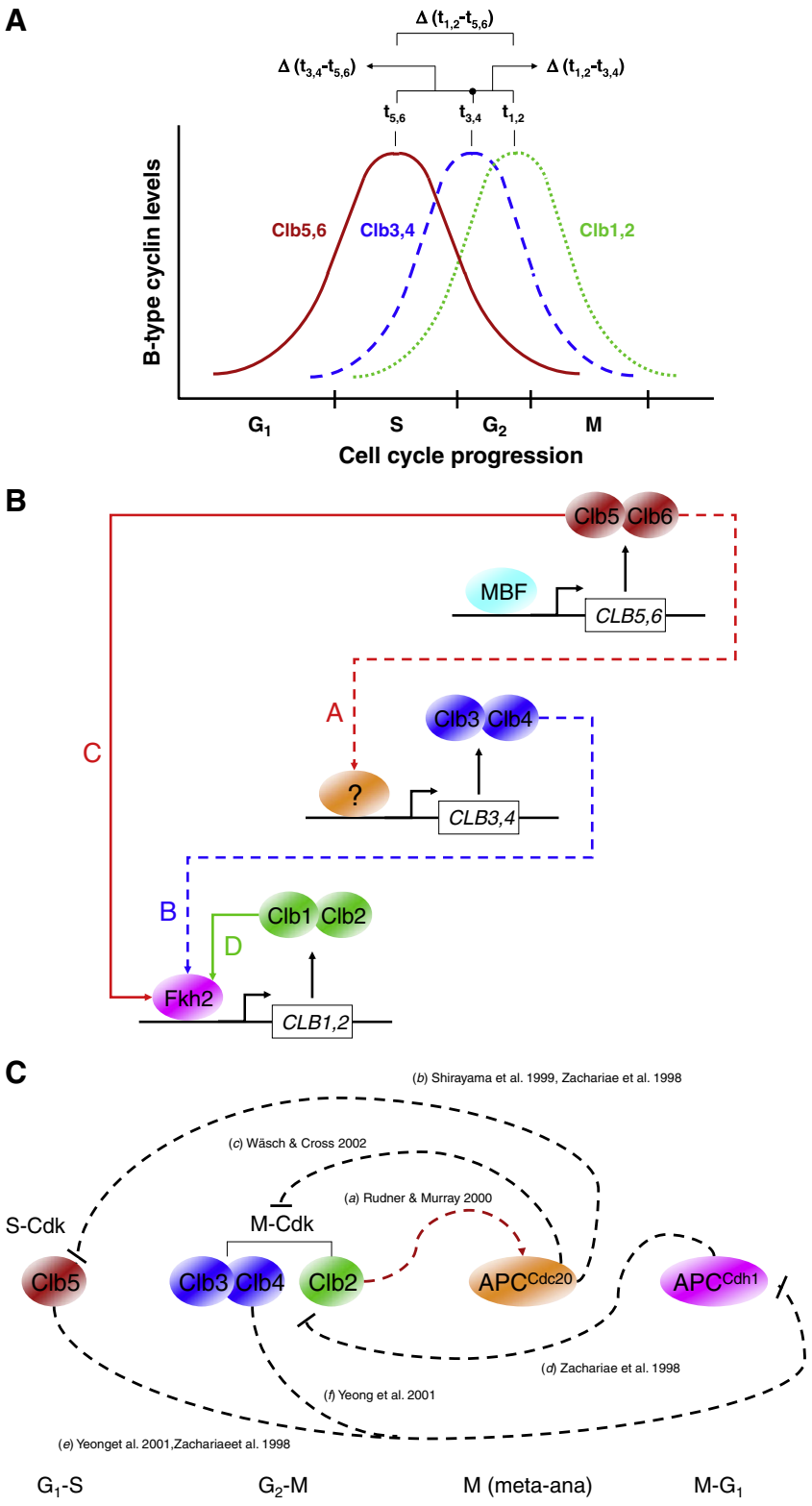

Fig. 1. Mechanisms of regulation of B-type cyclins. (A) Qualitative description of waves of Clbs during the cell cycle. Time distances between the peaks of Clbs, mentioned in the text, are also visualized: $t_{3,4}-t_{5,6}$ represents the time delay between Clb5,6 and Clb3,4, $t_{1,2}-t_{5,6}$ the time delay between Clb5,6 and Clb1,2 and $t_{1,2}-t_{3,4}$ the time delay between Clb3,4 and Clb1,2. (B) Transcriptional regulation of Clbs. Cdk1–Clb5,6 promote *CLB3,4* transcription (A), Cdk1–Clb3,4 promote *CLB1,2* transcription (B) together with Cdk1–Clb5,6 (C), and Cdk1–Clb1,2 promote *CLB1,2* transcription by stimulating its own production (D). For simplicity, Cdk1 subunit has been omitted. See text for details. (C) Clb-regulated degradation. Phosphorylation of Cdc20 by Cdk1–Clb2 activates APC^{Cdc20} (*a*). APC^{Cdc20} targets Clb5 for degradation (*b*) and degrades also mitotic cyclins (*c*). APC^{Cdh1} degrades Clb2 further during mitotic exit and in the following G_1 phase (*d*). Phosphorylation of Cdh1 by Cdk1–Clb5 (*e*) and by Cdk1-Clb3,4 (*f*) inactivates APC^{Cdh1}, thereby permitting the subsequent accumulation of Clb2. Bibliographic references are also indicated.