Supporting Text1

Stable stochastic dynamics in yeast cell cycle

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In this Supporting Text, reactions involved in Fig.1 of the main text are explained. Fig.1 contains reactions among 13 genes, 13 mRNAs, and 53 chemical states of proteins and protein complexes. Although all mRNAs and all forms of proteins are assumed to be degraded with certain specific rates in the model, explanation of those degradation processes is omitted in the following description. For figures of chemical schemes inserted in this Supporting Text, protein A in the chemical state *X* is denoted by A*X*. Catalytic actions are denoted by dotted arrows. Active proteins are denoted in red. The unstable short-lived protein is underlined.

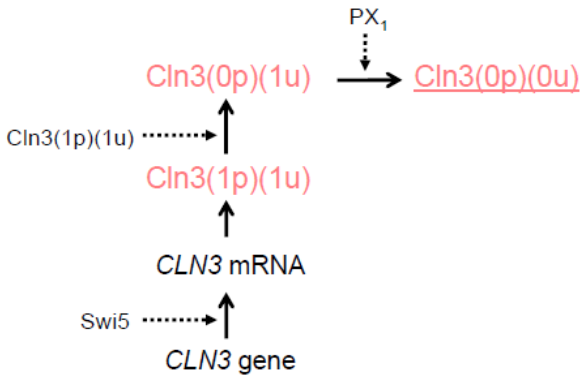
1) Cln3

Experimental observations

The *CLN3* promoter contains ECB (early cell cycle box) and the Swi5 binding site [1, 2]. Although the *CLN3* mRNA level increases three- to four-fold at around the M-G1 boundary [3], the Cln3 protein level is kept low and oscillation of the Cln3 level is modest throughout the cell cycle [4]. Cln3 localizes to nucleus [5] and forms Cln3/Cdc28 complex. The phosphorylated Cln3 is ubiquitinated in a Cdc34-dependent manner [6-8] and the ubiquitinated Cln3 is highly unstable with a half-life time of ~10 min [4, 8].

Model

The *CLN3* expression is assumed to be regulated by the transcriptional activator Swi5. We assume the complex, Cln3/Cdc28, autophosphorylates itself. PX_1 represents the ubiquitin ligase working on Cln3, whose abundance is assumed to be constant in the model. Thus, the phosphorylated Cln3 denoted by Cln3(0p)(1u) is ubiquitinated with a constant rate. All forms of Cln3 can work on SBF and MBF during stage1.



2) SBF

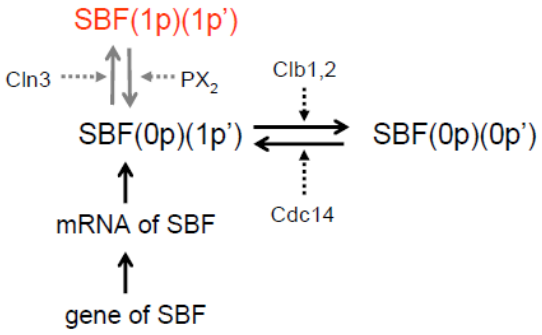
Experimental observations

SBF (SCB binding factor) is a transcriptional activator composed of Swi4 and Swi6, and binds to the SCB sequence in the form of a heterodimer [9]. Abundance of SBF changes through the cell cycle partially because of the fluctuation in the *SWI4* mRNA level, but this change is not much correlated to its ability to regulate the *CLN2* transcription [10]. Prior to late G1, SBF binds to the SCB promoter, but Whi5 binds to SBF at the promoter and inhibits the SBF activity. In late G1, Cln3/Cdc28 promotes dissociation of Whi5 from SBF at the promoter and thereby SBF recovers its activity [11]. In G2-M phase, SBF dissociates from the promoter when Swi4 is phosphorylated by Clb1,2/Cdc28 [10, 12]. The nuclear localization of Swi6 is regulated in a cell cycle dependent manner [13], whereas the DNA binding component, Swi4, remains in nucleolus throughout the cell cycle [14]. Phosphorylation of Swi6 by cyclin/Cdc28 at the end of G1 prevents nuclear localization of Swi6. In late M, Cdc14 is released from nucleolus and dephosphorylates Swi6, which leads to the accumulation of Swi6 in nucleus [13].

Model

We treat the SBF complex as a single unit and do not take account of its individual components separately. SBF is assumed to be constantly produced from the putative SBF gene and its mRNA. SBF has two symbolic phosphorylation sites denoted by (αp) and (α'p'). While (αp) represents change of the chemical state in the G1/S transition, (α'p') represents that in G2 and M phases. α is turned to be 1 by the action of Cln3/Cdc28 but the period that Cln3/Cdc28 is active is limited only to stage1. PX_2 represents the hypothetical inactivator of SBF(1p)(1p'), whose amount is assumed to be

constant throughout the cell cycle. Reactions on (α'p') represent changes in both Swi4 and Swi6. We assume phosphorylation of Swi6 is carried out mainly by Clb1,2/Cdc28 rather than by Clb5,6/Cdc28, so that the (α'p')-site is phosphorylated by Clb1,2/Cdc28 and dephosphorylated by Cdc14.



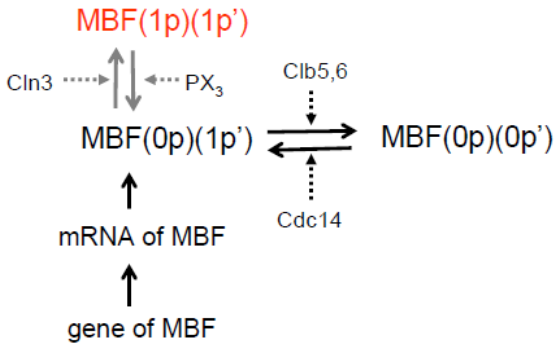
3) MBF

Experimental observations

MBF (MCB binding factor) is a transcriptional activator composed of Mbp1 and Swi6, which binds to the MCB sequence in the form of a single heterodimer [9]. Not much is know about the regulation of Mbp1 in the MBF complex. Swi6 is regulated as in the case of SBF complex.

Model

The model for molecular interactions of MBF is similar to that of SBF. We assume that the MBF complex is produced from the putative MBF gene and mRNA. MBF is assumed to have two reaction sites as in the case of SBF, but the (α'p')-site is phosphorylated by Clb5,6/Cdc28 instead of Clb1,2/Cdc28.



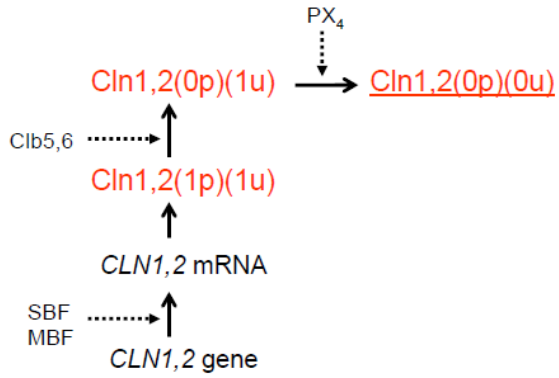
4) Cln1,2

Experimental observations

Expression of *CLN1* and *CLN2* is regulated by the MCB (MluI cell cycle box) and SCB (Swi4,6-depnendent cell cycle box) promoters, and the MCB and SCB promoters are activated by MBF and SBF, respectively [2, 15-17]. Clb6/Cdc28 negatively regulates the Cln2 function at the protein level [18]: Cdc28 phosphorylates both Cln1 and Cln2, and the phosphorylated Cln1 and Cln2 are ubiquitinated by SCF^{Grr1} [19-21]. The ubiquitinated Cln1 and Cln2 are rapidly degraded with half-life time of 8-10 min [4, 21]. Cln2 can also form Cln2/Cdc28 complex even when Cln2 is phosphorylted by Cdc28 [21]. Although Cln2 is found at similar concentrations in cytoplasm and nucleus [22], the hypophosphorylated Cln2/Cdc28 is mainly in nucleus and the phosphorylated Cln2/Cdc28 is localized to cytoplasm [5, 23].

Model

Both SBF and MBF activate the expression of *CLN1,2*. Cln1,2 is assumed to have two reaction sites, (αp) and (αu). Clb5,6/Cdc28 phosphorylates the (αp)-site of Cln1,2, and the phosphorylated form of Cln1,2 is ubiquitinated. PX_4 represents the ubiquitin ligase activity of SCF^{Grr1}, whose abundance is assumed to be constant.



5) Sic1

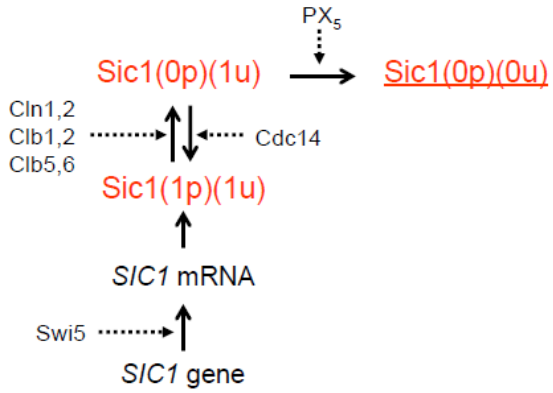
Experimental observations

The *SIC1* promoter is activated by Swi5 and its expression increases three- to four-fold around the M-G1 boundary [24-26]. Sic1 is distributed in both cytoplasm and nucleus at similar concentrations [22], and it inhibits the Clb5/Cdc28 kinase activity during G1 by forming the ternary complex with Clb5/Cdc28 [26]. Abundance of Clb5 begins to

increase at the G1-S transition, and when Clb5 exists in excess, Clb5/Cdc28 phosphorylates Sic1 [20]. Cln2/Cdc28 also phosphorylates both the monomeric Sic1 and Sic1 in the Sic1/Clb5/Cdc28 ternary complex [20, 27]. When either form of Sic1 is phosphorylated on at least six out of nine CDK sites, it is recognized and ubiquitinated by SCF^{Cdc34} [20, 25-28]. Sic1 is unstable in S phase with a half-life of 10 min or less [29] and its abundance is low before the M-G1 boundary. Swi5 activates the *SIC1* expression and Cdc14 desphorylates Sic1 to avoid ubiquitination [30, 31].

Model

Transcription of *SIC1* is activated by Swi5. Sic1 is assumed to have two reaction sites, (αp) and (αu). Cln1,2, Clb1,2, and Clb5,6 phosphorylate the (αp)-site of Sic1, which is in turn dephosphorylated by Cdc14. The phoshorylated form of Sic1, Sic1(0p)(1u), is ubiquitinated in proportion to its abundance. PX_5 represents the constant ubiquitin ligase activity of SCF^{Cdc34}. All forms of Sic1 proteins can bind to Clb1,2/Cdc28 and Clb5,6/Cdc28.



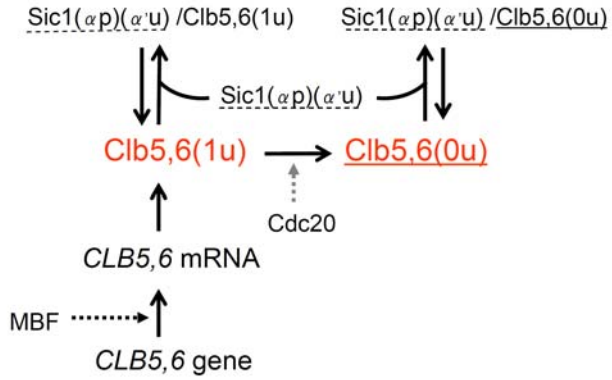
6) Clb5,6

Experimental observations

CLB5 mRNA is very rare in early G1 and accumulates to high level, and then rapidly decreases in G2 [26]. MBF is a potential activator of *CLB5* and *CLB6*, which shows high affinity to their promoters in microarray experiments [2, 15]. During G1, abundance of Clb5/Cdc28 is low and its activity is inhibited by the association with Sic1. Clb5/Cdc28 accumulates in nucleus to increase its activity as cell enters S phase, but APC^{Cdc20} leads to its sudden decrease at the metaphase-anaphase transition [26, 32]. Half-life of Clb5 is 5-10 min in G1 and 15-20 min in S and M [33].

Model

Expression of *CLB5,6* is positively regulated by MBF. Clb5,6 has a reaction site which can be ubiquitinated by Cdc20 in stage3-5. Kinase activity of Clb5,6/Cdc28 is inhibited when bound to Sic1 and the activity is recovered when Sic1 in the ternary complex is degraded.



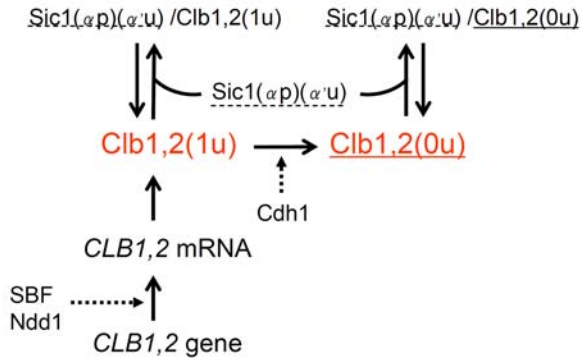
7) Clb1,2

Experimental observations

Transcription of *CLB2* is activated by Mcm1/Fkh2/Ndd1 during G2 and M. The microarray analyses suggest that SBF is another activator of *CLB2* [2, 16]. Clb2 is strongly localized in nucleus at all stages of cell cycle [34], but its abundance is regulated by both transcriptional activation and APC^{Cdh1}-mediated ubiquitination. During G1 phase, when the APC^{Cdh1} level is high, Clb2 is highly unstable and barely detected. The Clb2 level begins to increase in S phase, peaks during M phase, and declines at some time in late anaphase [35-39]. Clb2 is stable during S and M with half-life of > 1h, but extremely short-lived in G1 with half-life of < 5 min [33, 40].

Model

Expression of *CLB1,2* is activated by both SBF and Ndd1. Clb1,2 is ubiquitinated by Cdh1. Clb1,2/Cdc28 is inactivated by forming a complex with Sic1 and is activated when Sic1 in the complex is degraded.



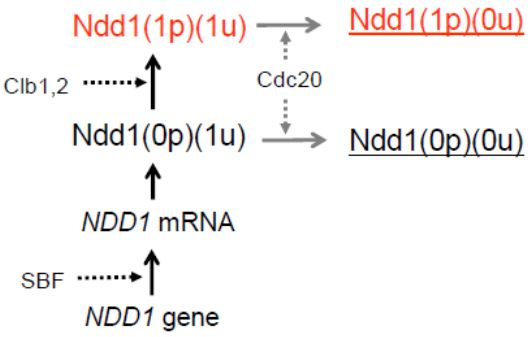
8) Ndd1

Experimental observations

SBF binds to and activates the *NDD1* promoter [2, 15]. Ndd1 is localized in nucleus [36] forms the Mcm1/Fkh2/Ndd1 ternary complex. The Mcm1/Fkh2 complex occupies *CLB2* and *SWI5* promoters throughout the cell cycle [41] and these promoters are activated when Ndd1 is recruited [42, 43]. For this recruitment, phosphorylation of Ndd1 by Clb2/Cdc28 is required. Ndd1 begins to decrease at the beginning of disassembly of the mitotic spindles and remains unstable until anaphase spindles disappear [36].

Model

Expression of *NDD1* is activated by SBF. We assume Ndd1 is ubiquitinated by Cdc20 and it has two reaction sites, (αp) and (αu). The former is phosphorylated by Clb1,2/Cdc28 to be active, and the latter is ubiquitinated by Cdc20 during stage3-5.



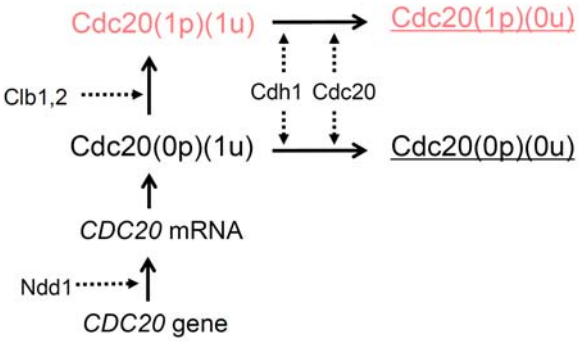
9) Cdc20

Experimental observations

Expression of *CDC20* is activated by the Mcm1/Fkh2/Ndd1 complex and others [2, 16], leading to the oscillation of the *CDC20* mRNA level which peaks in M phase [38]. The abundance of Cdc20 fluctuates throughout the cell cycle, rising in S phase, being maximal during M phase, and declining on exit from M phase [37, 38, 44]. Cdc20 is localized to nucleus [44] and the spindle checkpoint keeps the Cdc20 level low until all kinetochores attach to spindle microtubules. The check-point induced Cdc20 degradation requires the physical interaction between Cdc20 and Mad2 and involves APC [45]. Cdh1 is not required for this process [45], but APC^{Cdh1} contributes to the degradation of Cdc20 in late G1 [46]. In this manner Cdc20 is unstable throughout the cell cycle with half-life time of < 3 min during S, G2, and early M and is less stable in anaphase and G1 [38]. Activity of Cdc20 is also regulated by the spindle checkpoint. Spindle checkpoint proteins, Mad2 and Mad3, bind to Cdc20 to prevent it from activating APC until the metaphase-anaphase transition [45]. Phosphorylation of the APC core subunits by Clb2/Cdc28 enhances the association of Cdc20 with APC and increases the APC^{Cdc20} activity [47, 48].

Model

Expression of *CDC20* is activated by Ndd1. It is experimentally known that phosphorylation of APC by Clb2/Cdc28 promotes APC^{Cdc20} activity, and this effect is represented as phosphorylation and activation of Cdc20 by Clb2/Cdc28 in the model. In order to include the effect of the checkpoint-induced Cdc20 degradation into the model, we assume that Cdc20 in the model autoubiquitinates itself throughout the cell cycle. The checkpoint represses the Cdc20 activity until the metaphase-anaphase transition takes place. We express this checkpoint mechanism by imposing the condition that Cdc20 works on the target proteins other than itself only during stage3-5: Cdc20-dependent ubiquitination of Clb5,6, Ndd1, and Pds1 is limited to stage3-5.



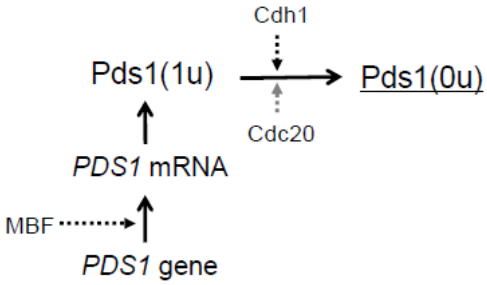
10) Pds1

Experimental observations

The *PDS1* mRNA level fluctuates in a cell cycle-dependent manner with maximal accumulation around the G1-S transition [49]. The microarray analysis showed that MBF binds to the *PDS1* promoter, suggesting that the expression of *PDS1* is activated by MBF [15]. Pds1 is localized in nuclear [50] and both its productivity and stability are regulated during cell cycle. At the end of metaphase, Pds1 is ubiquitinated by APC^{Cdc20} and thereby undergoes rapid degradation [39, 47, 50, 51]. Pds1 is also targeted by APC^{Cdch1} and is highly unstable during anaphase and G1 (half-life <15 min) [48, 51]. In consequence, Pds1 exists during the period from late G1 or from early S to the metaphase-anaphase transition [50]. The anaphase inhibitor Pds1 (securin) binds to Esp1 (separin) and inhibits the activity of Esp1. The rapid degradation of Pds1 at the metaphase-anaphase transition is required for the liberation of Eps1. When released form Pds1, Eps1 can induce cleavage of the cohesion complex that holds sister chromatids together.

Model

Expression of *PDS1* is activated by MBF. Pds1 is ubiquitinated by APC^{Cdc20} (during stage3-5) and by APC^{Cdch1}.



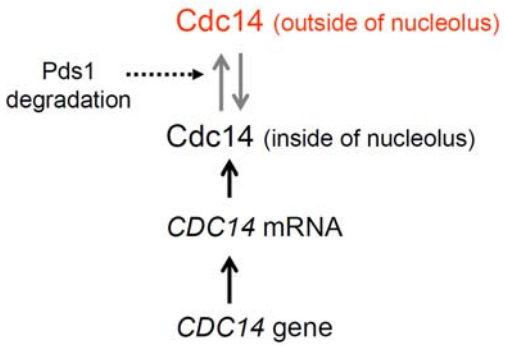
11) Cdc14

Experimental observations

Although the Cdc14 level is roughly constant, the subcellular localization of Cdc14 changes remarkably in a cell cycle-dependent manner. From G1 to early M phase, Cdc14 is localized in nucleolus as a part of the RENT complex, which prevents Cdc14 from phosphorylating its target proteins. Cdc14 is released from the RENT complex at some time in anaphase [35, 52]. Release of Cdc14 requires degradation of Pds1 by APC^{Cdc20} [32]. The released Cdc14 spreads throughout nucleus and cytoplasm and it dephosphorylates Cdh1, Swi5, and Sic1 to promote exit from mitosis [30, 31, 53, 54]. Then, Cdc14 comes back into nucleolus as cell enters G1 phase [35]. The localization of Cdc14 is regulated by proteins which are not included in the present model.

Model

Cdc14 is distinguished by its location. Localization is regulated by changing the rates of exporting and importing Cdc14 from and to nucleolus in a stage-dependent manner: During stage4, the exporting rate of Cdc14 is $r_{ex} = (\ln 2/160)(\Delta n)n_{in}$, where n_{in} is the number of Cdc14 locating inside of nucleolus and Δn is the number of Pds1 molecules degraded during stage3, and the importing rate of Cdc14 is $r_{im} = 0.2(\ln 2/10)n_{out}$, where n_{out} is the number of Cdc14 locating outside of nucleolus. During other stages, $r_{ex} = 0.2(\ln 2/160)n_{in}$ and $r_{im} = (\ln 2/10)n_{out}$.



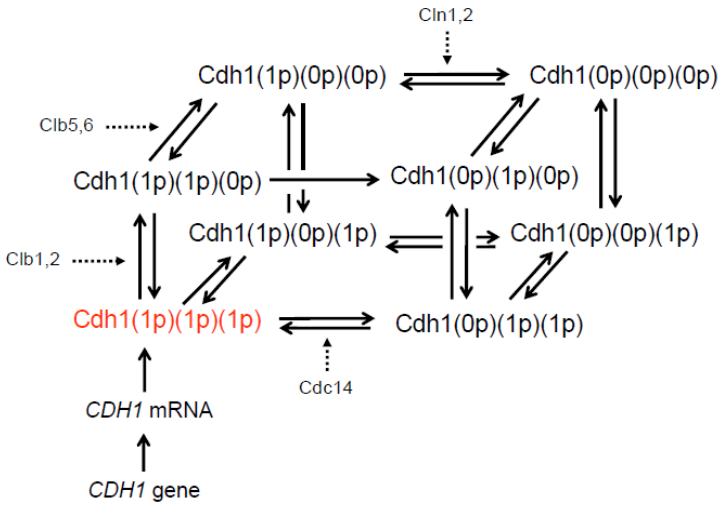
12) Cdh1

Experimental observations

Abundance of Cdh1, as well as that of the *CDH1* mRNA, are roughly constant during cell cycle [37, 38], but the activity of APC^{Cdch1} is regulated by cyclin/Cdc28 complexes through phosphorylation of Cdc14. During S, G2, and M phases, Cdc28 associated with Cln1, Cln2, Clb1, and Clb5, phosphorylates multiple sites of Cdh1 [44, 46, 47, 55]. The phosphorylated Cdh1 do not bind to APC and is exported into cytoplasm [44]. Activity of APC^{Cdch1} is restored, when Cdh1 is dephosphorylated by Cdc14 at the end of mitosis [53].

Model

We assume Cdh1 has three reaction sites, each of which is phosphorylated by a single kind of cyclin/Cdc28. Namely, Cln1,2, Clb1,2 and Clb5,6 respectively work on different sites of Cdh1. All these reaction sites are dephosphorylated by Cdc14 independently. Among eight forms of Cdh1, only Cdh1(1p)(1p)(1p) is assumed to be active.



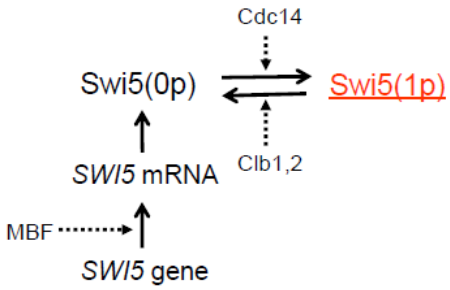
13) Swi5

Experimental observations

Transcription of *SWI5* is specific to G2 and M phases. Swi5 is the transcriptional factor of Sic1 and Cln3, and the activity of Swi5 is regulated by its localization. Prior to anaphase, Cdc28 mediates the Swi5 localization in cytoplasm [54, 56]. Around the anaphase-telophase boundary, Swi5 is dephosphorylated by Cdc14 and accumulates in nucleus [54, 57]. Swi5 is highly unstable in the nucleus and the majority of Swi5 is degraded by the time of cell separation [57].

Model

Expression of *SWI5* is activated by MBF. Swi5 is phosphorylated by Clb1,2/Cdc28 and dephosphorylated by Cdc14. The phosphorylated form of Swi5 is assumed to localize in cytoplasm and hence be inactive. The dephosphorylated form of Swi5, on the other hand, is assumed to localize in nucleus, where it can activate the transcription of *SIC1* and *CLN3*. We assume dephosphorylated form of Swi5 is rapidly degraded with the same half-life time of ubiquitinated proteins because Swi5 is highly unstable in nucleus.



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