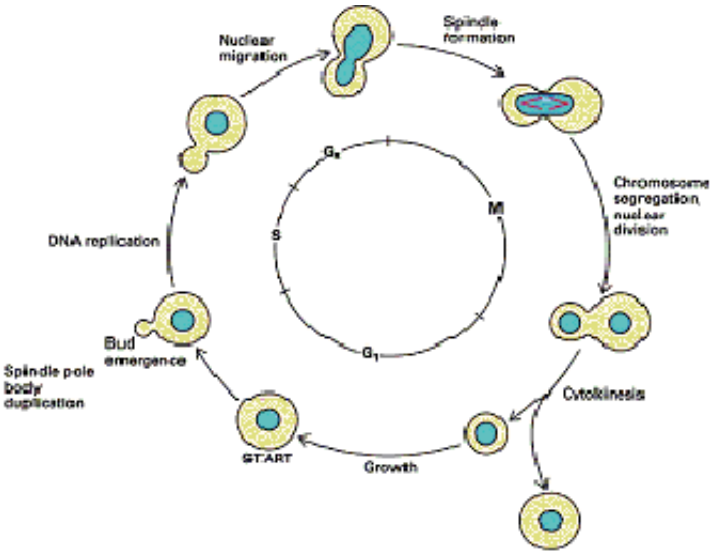
Introduction

Biology of the budding yeast:

The cell cycle is the succession of events whereby a cell grows and divides into two daughter cells that each contain the information and machinery necessary to repeat the process. Between one cell division and the next, all essential components of the cell must be duplicated. The most important component is the genetic material (DNA molecules present in chromosomes), which must be accurately replicated and the two copies carefully segregated to the two daughter cells. The processes of DNA replication and sister chromatid separation occur in temporally distinct phases of the eukaryotic cell cycle. These are known as S-phase (DNA synthesis) and M-phase (mitosis), In general, S and M phases separated by two gaps, known as G1 and G2.



The unicellular budding yeast, *Saccharomyces cerevisiae*, is a model system to study cell cycle regulation. As a yeast cell progresses through the cell cycle, it halts at two major checkpoints:

- **the G1 checkpoint**: If DNA damage is detected, mating pheromone is present, or the cell has not reached the critical size, the cell arrests in G1 and is unable to undergo the Start transition which commits the cell to a new round of DNA synthesis and mitosis.
- **the spindle assembly checkpoint**: If DNA damage is detected, DNA is not replicated completely, or chromosomes are not aligned on the metaphase plate, the cell arrests in metaphase and is unable to undergo the Finish transition, whereby sister chromatids are separated and the cell divides.

These checkpoints are enforced by **the Cdk/cyclin complexes**, a family of protein kinases. The catalytic subunit of these complexes, the cyclin-dependent kinase (Cdk), is only active when combined with a regulatory cyclin subunit. In budding yeast, there is only one Cdk (called Cdc28); and nine different cyclins (Cln1-3, Clb1-6). Depending on the cyclin partner, Cdc28/cyclin dimers accomplish specific and different tasks. Proper progression through the cell cycle requires the successive activation and inac-

tivation of these Cdc28/cyclin dimers. There are several different mechanisms for regulating Cdc28 activity in the cell, namely:

- through the synthesis of cyclins by various transcription factors (SBF, MBF and Mcm1).
- through the degradation of cyclins (promoted by Cdc20/APC, Cdh1/APC, and Grr1/SCF).
- through association with stoichiometric CDK inhibitors (Sic1 and Cdc6, and Far1).
- through phosphorylation and dephosphorylation of Cdc28 by Swe1 and Mih1.

The regulatory proteins that control the activities of Cdc28 and ensure the proper progression of cell cycle events are listed below.

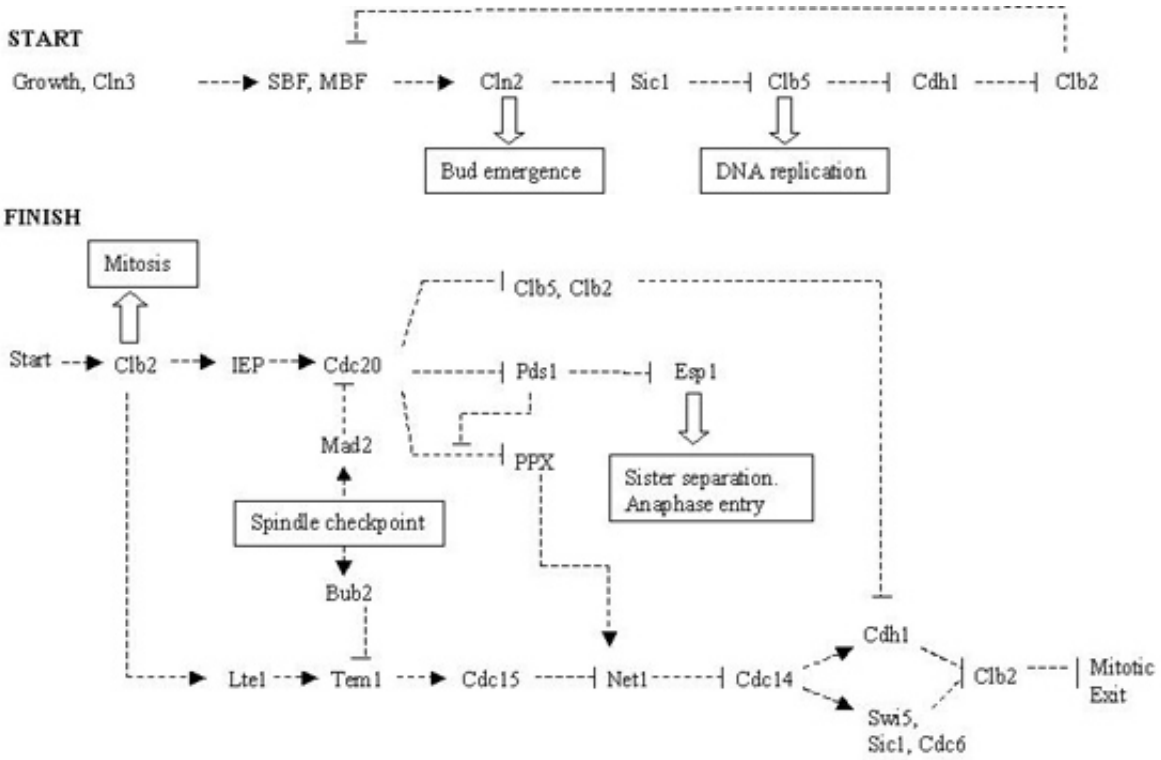
The components of the model and their function

The regulatory proteins that control the activities of Cdc28 and ensure the proper progression of cell cycle events are listed below.

Cdc28	Cyclin-dependent kinase.
Cln3	G1-cyclins initiating Start events.
Bck2	Protein initiating Start events.
Cln1,2	Cyclins involved in budding (represented as Cln2 in the model).
Clb5,6	B-type cyclins appearing late in G1, involved in DNA synthesis (represented as Clb5 in the model).
Clb1,2	B-type cyclin essential for mitosis, present in S/G2/M phase (represented as Clb2 in the model).
Sic1	Stoichiometric inhibitor of Cdc28/Clb2 and Cdc28/Clb5.
Cdc6	Stoichiometric inhibitor of Cdc28/Clb2. Also a licencing factor for DNA replication.
APC	Anaphase Promoting Complex, a multi-enzyme complex reponsible for the degradation of Clb2, Clb5, and Pds1, and it requires Cdc20 or Cdh1 as a cofactor.
IE	Intermediary enzyme, a hypothetical protein involved in activating Cdc20 in the model, now identified as the phosphorylated form of the APC core.
Cdc20	Activator of the APC; protein involved in Clb2, Clb5 and Pds1 proteolysis, and required for exit from mitosis.
Cdh1	Activator of the APC; protein involved in Clb2 and Pds1

	proteolysis.
Pds1	Stoichiometric inhibitor of Esp1 that prevents sister chromatid separation.
Esp1	Separin protein required for sister chromatid separation.
PPX	Hypothetical, Pds1-inhibitable phosphatase that keeps Net1 unphosphorylated, thereby sequestering Cdc14 in the nucleolus, functionally equivalent to the "opposite of FEAR".
Mad2	"Mitosis Arrest Deficient" -- checkpoint protein that keeps Cdc20 inactive until the chromosomes are properly aligned.
Bub2	"Budding Uninhibited by Benomyl" -- checkpoint protein governed by spindle orientation.
Lte1	GTP-exchange factor, present in the bud, and an activator of Tem1.
Tem1	GTP-binding protein and a component of the MEN pathway.
Cdc15	Kinase essential for late nuclear division and a component of the MEN pathway.
Net1	Nucleolar protein and a stoichiometric inhibitor of Cdc14.
Cdc14	Phosphatase required for exit of mitosis.
Swi5	Transcription factor for Sic1 and Cdc6.
SBF	Transcription factor for Cln2.
Mcm1	Transcription factor for Clb2, Cdc20 and Swi5.
MBF	Transcription factor for Clb5.

Schematic diagram of cell cycle progression:

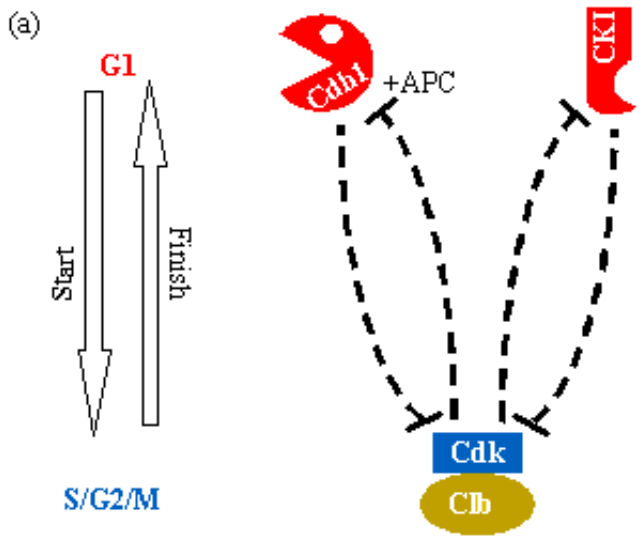


Basic mechanism

To understand the basic logic of the cell cycle, to a first approximation, we (and, independently, Kim Nasmyth) have envisioned that the cell cycle in budding yeast is an alternation between **two self-maintaining stable steady states** (G1 and S/G2/M). The Start transition carries a cell from G1 to S/G2/M, and the Finish transition from M back to G1 (Nasmyth, 1996, Tyson et al., 1995, Tyson et al., 2001).

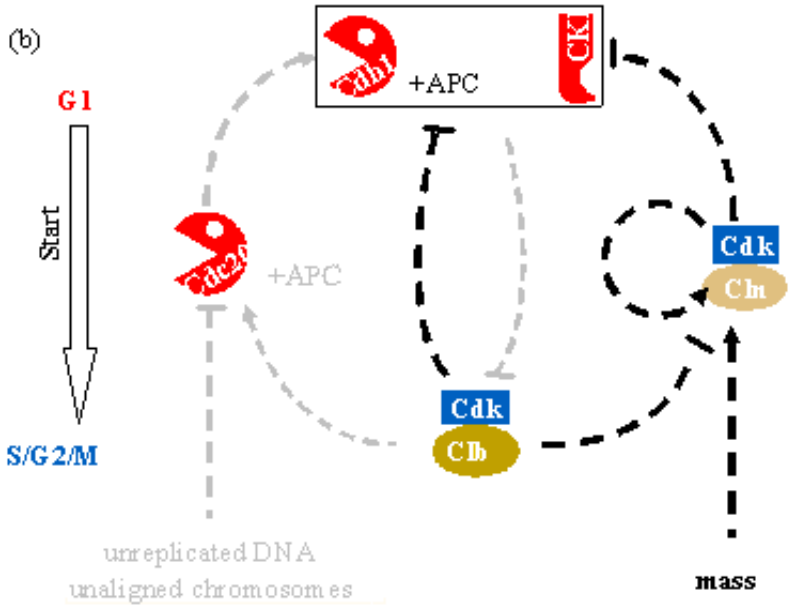
The two self-maintaining steady states arise primarily from the **mutual antagonism** between B-type cyclins (Clb1-6, in association with Cdc28) and the G1 stabilizers (Cdh1, Sic1 and Cdc6). Cdh1/APC degrades the Clbs, whereas Sic1 and Cdc6, referred to together as the CKIs, stochiometrically inhibit Cdc28/Clb complexes. Clb-kinases, on the other hand, can inactivate Cdh1 and destabilize CKIs. Since Clb-kinases and the G1 stabilizers mutually inhibit each other, these two classes of proteins cannot coexist.In the G1 state, Clb-kinase activities are low because Clb synthesis processes are turned OFF, their degradation by APC/Cdh1 is ON, and their inhibitors, the CKIs, are abundant. The reverse is true in the S/G2/M phase. Furthermore, it is important to note that:

a. The transitions between these two alternative steady states (G1 and S/G2/M) requires helper molecules (detailed in Chen et al., 2000).

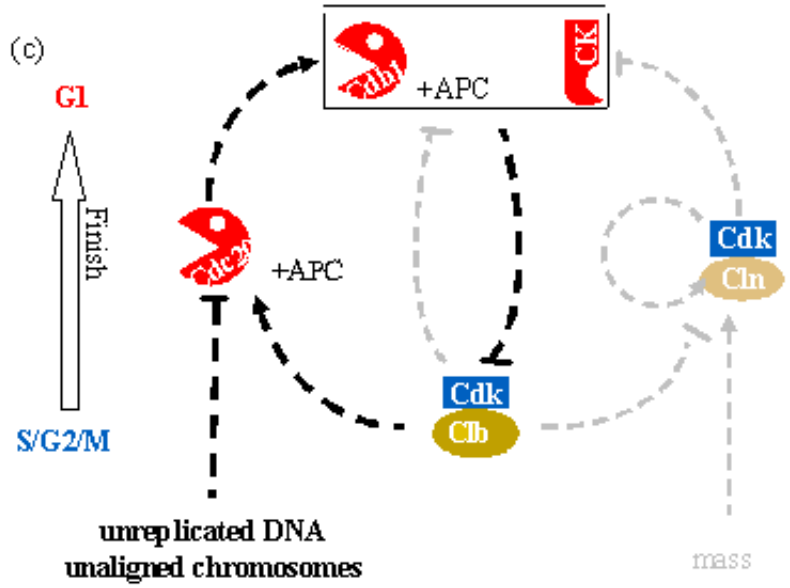


b. The Start transition is facilitated by Cln-kinases (Cln1-3/Cdc28 complexes) that can phosphorylate and inactivate CKI and Cdh1, but are not themselves opposed by CKI and Cdh1. This transition is driven by cell growth. When the small

daughter cell has grown to a critical size and Cln-kinase activities have reached a critical level, CKI and Cdh1 are inactivated, Clb-kinase activities increase, a bud emerges, DNA replication commences and spindle pole is duplicated. (The mother cell executes Start soon after birth because it has already attained the critical size.) The rising activity of Clb-kinases turns off Cln synthesis, causing Cln-kinase activities to drop in preparation for the Finish transition.



c. The Finish transition is facilitated by Cdc20, which is activated indirectly by Clb-kinases. When the spindle assembly checkpoint is lifted (DNA synthesis is complete and chromosomes are aligned on the metaphase plate), Cdc20 is activated, sister chromatids are separated, and Clbs are partially degraded. Cdc20 also initiates the activation of the phosphatase Cdc14, which reverses the inhibitory effects of Clb-kinases on Cdh1 and CKIs, allowing the latter two to overpower the Clb-kinases and extinguish their activities. As Clb-kinase activities drop after Finish, Cdc20 activity also disappears, preparing the cell for the subsequent Start transition.



INTRODUCTION

Major improvement of the model:

The previous model of the budding yeast (Chen et al., 2000) gives an adequate description of the Start transition, but, since it was published, many more molecular details about the Finish transition have come to light. Also in that paper, Clb2-kinase was assumed to activate Cdc20 directly, making the checkpoint protein Mad2 essential for cell viability, which is contrary to observation.

Here we introduce an intermediary enzyme, IE, that provides a time delay between Clb2 activation and Cdc20 activation, such that Mad2 is no longer an essential protein. The new model also accounts for how the MEN pathway facilitates Cdc14 release from the RENT complex and how the spindle assembly checkpoint impinge on the cell cycle engine.