<u>Cell Cycle Vignettes</u>

The Cell Cycle of Budding Yeast

John J. Tyson, Katherine C. Chen & Bela Novak
Department of Biological Sciences
Virginia Polytechnic Institute & State University
Blacksburg, Virginia USA
&
Centre for Integrative Systems Biology
Oxford University, Oxford UK

The cell cycle of budding yeast has become a hallmark problem of molecular systems biology for a number of reasons. (1) The cycle of DNA replication, mitosis and cell division is crucial to all aspects of biological growth, development and reproduction. (2) The genes and pathways for cell cycle control seem to be very similar in all eukaryotes, including mammals. (3) Budding yeast is exceptionally good for genetic analysis of cell cycle controls because it can proliferate as haploid cells, and its genetic makeup can be easily altered by standard tools of molecular genetics. For this reason, a great deal is known about the molecular machinery regulating the events of the budding yeast cell cycle. (4) The machinery is very complicated, and its properties cannot be worked out reliably by intuitive reasoning alone. (5) Comprehensive mathematical models of the budding yeast cell cycle have been built and tested against a wide range of experimental observations. (6) The models make interesting predictions that have been confirmed, in large part, by experimental tests.

Physiology

Budding yeast gets its name from its unusual style of asymmetric division into a large mother cell and a small daughter cell (Pringle and Hartwell 1981). After a G1 period, the budding yeast cell initiates a new bud at about the same time that it enters S phase (DNA synthesis). Also, at this time, the yeast cell replicates its spindle pole bodies and begins preparations for mitosis. These cotemporaneous events of the budding yeast cell cycle are referred to as START. The bud first emerges from the cell in a burst of polarized growth but quickly switches to isotropic growth, to form an expanding spherical protrusion. Most of the net cell growth after this time goes into the bud. After DNA synthesis is finished, an intra-nuclear mitotic spindle is built and the replicated chromosomes are aligned at the metaphase plate. Simultaneously, the G2/M nucleus migrates to the neck between the mother and bud compartments and orients itself with one pole of the mitotic spindle in the mother cell and the other pole in the bud. During anaphase, the replicated chromosomes are partitioned into two groups: one group is pushed into the mother cell and the other into the bud. The stretched nucleus divides in two, and the cell separates at the bud neck to produce mother and daughter cells. The daughter cell generally has a considerably longer G1 period than the mother cell. It

must grow to a certain threshold size before it can initiate a new round of budding, DNA replication and division.

Progression through the budding yeast cell cycle is governed by a series of checkpoints. For instance, we have already mentioned that daughter cells must grow to a critical size before they can execute START. Also, DNA damage can delay execution of START until the damage is repaired. These cells will not execute the metaphase/anaphase transition until all chromosomes are fully replicated and properly aligned on the mitotic spindle (the 'spindle assembly checkpoint' or SAC). Budding yeast cells have a 'morphogenetic checkpoint' which blocks mitosis if the bud is not properly formed, and a spindle alignment checkpoint that blocks cell division if the daughter cell nucleus is not properly pushed into the bud compartment.

Molecular cell biology
Genetic analysis of the molecular machinery controlling the major events of the cell cycle was begun in budding yeast by Leland Hartwell, who identified dozens of genes that induced the 'cell division cycle' phenotype. By Hartwell's definition, a *CDC* gene is a gene that, when mutated, arrests all cells in a specific stage of the cell cycle. For example, *cdc7* mutant cells do not replicate their DNA or divide, but bud emergence and growth continue as normal. Mutant *cdc* genes are isolated as temperature-sensitive alleles that on replicate plates form colonies at 25°C but not at the restrictive temperature (36°C). Presumptive mutant cells on the high temperature plate must be examined microscopically to determine that the cells have uniformly arrested at a particular morphological stage of the cell cycle. Hartwell's first set of *CDC* genes defined two parallel pathways (Hartwell et al. 1974): one pathway relating to bud emergence, isotropic growth and nuclear migration, and the other relating to DNA replication and mitosis. These two pathways diverge at START and come together finally at mitotic exit. The gene with the earliest effect, whose mutation blocked cells before the START transition, was *CDC28*.

Hartwell's work set off a landslide of activity identifying *CDC* genes in a variety of organisms, finding other genes that interacted with *CDC* genes, and later cloning these genes and characterizing their protein products and the interactions among these proteins (for review, see Morgan 2007). Worthy of note was Paul Nurse's discovery a fission yeast gene, $cdc2^{+}$, which seemed to encode the same protein as *CDC28* in budding yeast. Nurse went on to show, quite unexpectedly, that a human gene, later called *CDK1*, encodes a protein that can rescue $cdc2^{-}$ mutant fission yeast cells. In 1989 a consortium of geneticists and biochemists demonstrated that MPF (mitosis promoting factor in frog eggs, a master controller for the progression of the cell cycle) is a dimer of a protein kinase, encoded by the $CDC28/cdc2^{+}/CDK1$ gene, and a regulatory subunit called cyclin B which had been identified years earlier by Tim Hunt. Because the cyclin subunit is required for activity of the protein kinase, the kinase is called cyclin-dependent kinase, or CDK for short. The discovery of the composition of MPF was the beginning of the modern era of molecular biology of cell cycle control in eukaryotic cells. In 2001 the Nobel Prize in Physiology or Medicine was shared by Hartwell, Nurse and Hunt.

In the 1990's a host of cell cycle genes were identified in budding yeast, including

CLN1,2: encoding G1 cyclins important for START and bud emergence.

CLB1-6: encoding B-type cyclins essential for DNA synthesis and mitosis.

CDC20: encoding a ubiquitin-ligase component essential for sister chromatid separation and cyclin degradation in anaphase.

CDC14: encoding a phosphatase essential for mitotic exit.

CDH1, SIC1: encoding proteins that stabilized G1 phase of the cell cycle.

The heart of the budding yeast cell cycle

Kim Nasmyth (1996) proposed to think of the budding yeast cell cycle as two alternative, self-maintaining states: **G1** (unreplicated chromosomes, growing but not committed to proliferate), and **S/G2/M** (committed to chromosome replication and mitosis). START is the transition from **G1** to **S/G2/M**, and mitotic exit is the transition from **S/G2/M** back to **G1**. The molecular correlates of these two states are **G1**: high activity of Cdh1 and Sic1, no kinase activity associated with Clb1-6; and **S/G2/M**: low activity of Cdh1 and Sic1, high Clb-dependent kinase activity. Finally, Nasmyth recognized that a possible reason for these alternative, self-maintaining states was the antagonism between Cdh1 and Sic1 on one side (destroying the activity of the Clb-dependent kinase complexes) and the B-type cyclins, Clb1-6, on the other side (inactivating Cdh1 and Sic1).

Nasmyth stopped short of identifying his idea with a bistable control system flipping back and forth between two stable steady states as cell-cycle-progression signals pushed the control system past saddle-node bifurcations. But this connection became the basis of a series of mathematical model of the budding yeast cell cycle control system developed by Novak, Tyson and coworkers (Chen et al. 2004; Tyson and Novak 2008) and by Alberghina et al. (2009). A brief overview of these models is presented in Fig. 1, and the interested reader is referred to the original literature for details.
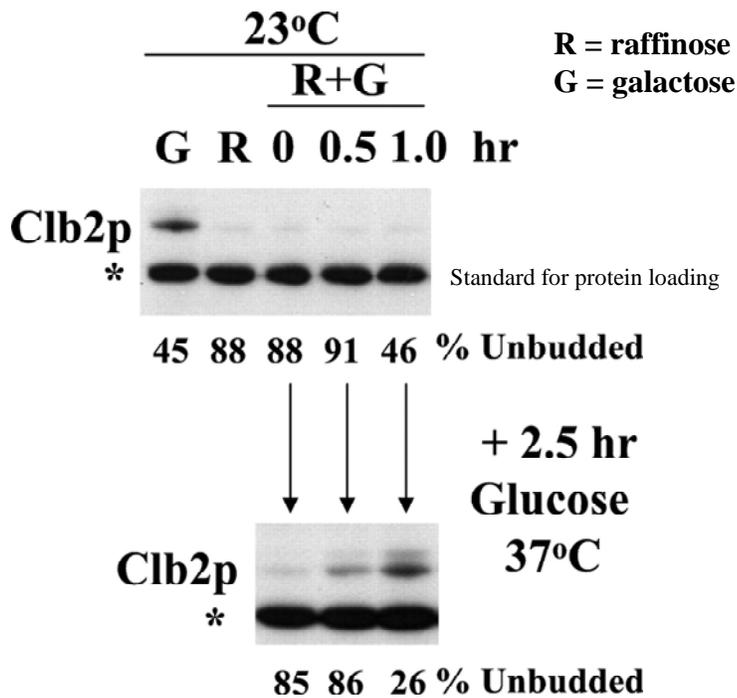
Experimental tests

Cross and collaborators (Cross et al. 2002) have confirmed experimentally a number of predictions of the Chen model, including the idea of two alternative stable steady states (see Fig. 2).

Web resources

Model webpage: http://mpf.biol.vt.edu/research/budding_yeast_model/pp/
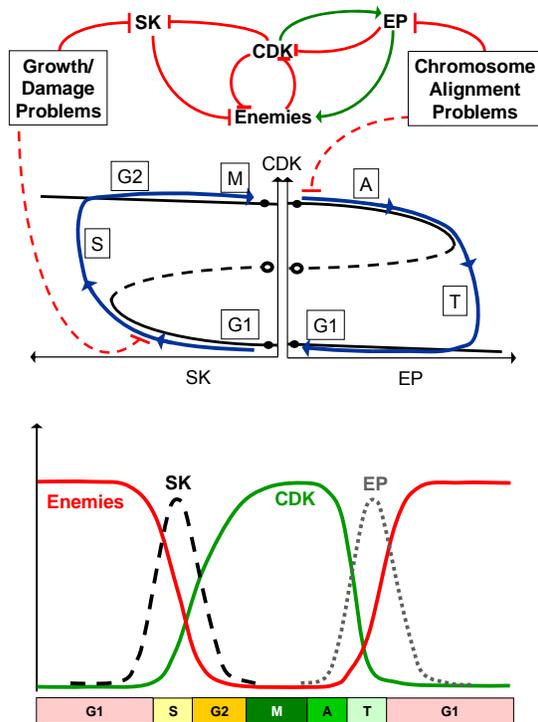
Figure 1. The heart of the budding yeast cell cycle. Adapted from Tyson & Novak (2008). (Top center) Cyclin-dependent kinase (CDK) and its enemies (Cdh1 and Sic1) are involved in an antagonistic (double negative) feedback loop, which can persist in either of two stable steady states: a G1-like state with low CDK activity and prominent enemy forces, and an M-like steady state with high CDK activity and the enemies in retreat. These two stable steady states are represented by black circles along the CDK axis (middle center). The pairs of circles side-by-side are meant to represent the same steady state under conditions when both the starter kinase (SK) and exit phosphatase (EP) are close to zero. The two stable steady states are separated by an unstable steady state (open circle). A newborn cell in G1 (middle left) can be induced to enter S/G2/M by activation of SK (Cln-dependent kinase), which phosphorylates and weakens Cdh1 and Sic1, allowing CDK activity to rise and trigger S phase (follow the blue arrow for the cell's passage through the cell cycle). Among other duties, CDK down-regulates SK, but the bistable switch remains in the upper state. Notice that, as SK activity increases, the stable G1-like steady state merges with and is annihilated by the unstable steady state at a saddle-node bifurcation. Exit from mitosis (middle right) is induced by EP (Cdc14 phosphatase), which activates the proteins that destroy CDK activity. When the upper steady state merges with the unstable steady state, the cell returns to the G1 state. Because EP activity depends on CDK, after CDK falls, EP activity also disappears, but now the enemies have the upper hand. The two blue arrows together form a 'hysteresis loop' that switches the CDK-control system from OFF to ON and back again during each cell cycle. (Bottom) Progression around the hysteresis loop corresponds to periodic changes in the activities of CDK, enemies, SK and EP. At START, a burst of SK activity flips the CDK switch ON, and at EXIT, a burst of EP activity flips the CDK switch OFF. (Top left and right) Checkpoints function by inhibiting either SK or EP. Growth requirements and damage repair processes typically halt the cell cycle in G1 phase by preventing up-regulation of SK activity. Misalignment of chromosomes on the spindle blocks exit from mitosis by preventing activation of EP.

23°C

R+G

G R 0 0.5 1.0 hr

Clb2p

*   Standard for protein loading

45 88 88 91 46 % Unbudded

+ 2.5 hr
Glucose
37°C

Clb2p

*

85 86 26 % Unbudded

R = raffinose
G = galactose

Figure 2. Experimental confirmation of alternative stable states with low or high Clb2-dependent kinase activity. Adapted from Cross et al. (2002). To test the prediction that a stable **G1** state of low Clb-dependent kinase activity coexists with a stable **S/G2/M** state of high Clb-dependent kinase activity when SK = EP = 0, Fred Cross and colleagues measured Clb2 abundance and cell-cycle phase in a mutant strain of budding yeast, *cln1Δ cln2Δ cln3Δ GAL-CLN3 cdc14^ts^*. When grown in raffinose or glucose, the *GAL* promoter is repressed, and the cells are unable to make any Cln proteins, so they have no Cln-dependent kinase activity (SK = 0 in Fig. 1). When grown at 37°C, this strain has no Cdc14 activity (EP = 0 in Fig. 1). In raffinose or glucose at 37°C, this strain has neither Cln3 nor Cdc14 activity, i.e., it is in the 'neutral' position in Fig. 1. In galactose-rich medium (*GAL* promoter active) at 23°C, the cells have both SK and EP, so they are able to progress around the cell cycle (the blue hysteresis loop in Fig. 1). Under these conditions, the cells proliferate asynchronously, as indicated (upper left) by the facts that 45% of the cells are unbudded (in G1) and the remainder of the cells are in S/G2/M with significant amounts of Clb2 protein. When this asynchronous population of cells is shifted to raffinose medium at 23°C for 6 h (i.e., deprived of SK), the cells arrest primarily in G1 phase (88% unbuddded) with un-detectable levels of Clb2 (lane R). This is the **G1** steady state in Fig. 1. Next, the culture is shifted to galactose + raffinose medium at 23°C to induce production of Cln3. At the end of the indicated periods of time, two aliquots of the culture are removed. One aliquot is analyzed immediately for bud index and Clb2 content (lanes 3, 4, 5 of upper gel); the other aliquot is shifted to glucose medium at 37°C for 2.5 h and then analyzed as before (lower gel). During the variable period of exposure to galactose, the cells produce increasing amounts of Cln3 protein (i.e., increasing pulses of SK in Fig. 1). Cells exposed to a small pulse of Cln3 (0 or 0.5 h in galactose) remained in the stable **G1** state, as indicated by lack of buds and little or no Clb2 protein (lanes 1 and 2 of lower gel). Cells that got a larger pulse of Cln3 (1 h in galactose) switched to the stable **S/G2/M** state (mostly budded cells with abundant Clb2—lane 3 of lower gel) and stayed there because EP was disabled at the higher temperature. The cells in all three lanes of the lower gel are exposed to the same conditions (SK = EP = 0), but the cells in lanes 1 and 2 are in the **G1** state, whereas the cells in lane 3 are in the **S/G2/M** state; i.e., the system is bistable.

5

References

Alberghina L, Coccetti P, Orlandi I (2009) Systems biology of the cell cycle of *Saccharomyces cerevisiae*: from network mining to system-level properties. Biotechnol Adv 27:960-978

Chen KC, Calzone L, Csikasz-Nagy A, Cross FR, Novak B, Tyson JJ (2004) Integrative analysis of cell cycle control in budding yeast. Mol Biol Cell 15:3841-3862

Cross FR, Archambault V, Miller M, Klovstad M (2002) Testing a mathematical model for the yeast cell cycle. Mol Biol Cell 13:52-70

Hartwell LH, Culotti J, Pringle JR, Reid BJ (1974) Genetic control of the cell division cycle in yeast. Science 183*:* 46-51

Morgan DO (2007) The Cell Cycle: Principles of Control. New Science Press, London

Nasmyth K (1996) At the heart of the budding yeast cell cycle. Trends Genet 12: 405-412

Pringle JR, Hartwell LH (1981) The Saccharomyces cerevisiae cell cycle. In: Strathern JN, Jones EW, Broach JR (eds) The molecular biology of the yeast Saccharomyces, Cold Spring Harbor Laboratory, Cold Spring Harbor, pp. 97-142

Tyson JJ, Novak B (2008) Temporal organization of the cell cycle. Curr Biol 18:R759-R768