The yeast cell cycle
The yeast cell cycle is controlled by a complex network of Cyclin/CDK.


An important factor that controls yeast division is the cell size.

The yeast cell cycle is controlled by a network of Cyclin/CDK.
At Start, the cell enters S phase and replicates its DNA. Start is triggered by a protein kinase, Cdk, whose activity depends on association with a cyclin subunit.

Cdk activity drives the cell through S phase, G2 phase, and up to metaphase. At the end of S phase, each chromosome consists of a pair of sister chromatids held together by tethering proteins.

After a gap (G2 phase), the cell enters mitosis (M phase), when the replicated chromosomes are aligned on the metaphase spindle, with sister chromatids attached by microtubules to opposite poles of the spindle.

Finish is accomplished by proteolytic machinery, APC, which destroys the tethers and cyclin molecules. The sister chromatids can be segregated to opposite sides of the cell (anaphase).

 Shortly thereafter the cell divides to produce two daughter cells in G1 phase.

(Chap 10 from Comput Cell Biol)
In G1 phase, APC is active and Cdk inactive, because it lacks a cyclin partner. At Start, the APC must be turned off so that cyclins may accumulate.

Cdk and APC are antagonistic proteins: APC destroys Cdk activity by degrading cyclin, and cyclin/Cdk dimers inactivate APC by phosphorylating some of its subunits. These major events of the cell cycle must be tightly regulated.

For instance, balanced growth and division is achieved in most cells by a size requirement for the Start transition. That is, cells must grow to a critical size before they can commit to chromosome replication and division. If this requirement is compromised by mutation, cells may become morbidly large or small.

A second crucial regulatory constraint is to hold off the Finish transition if there have been any problems with DNA replication or chromosome alignment. Were anaphase to commence under such conditions, then daughter nuclei would not receive a full complement of chromosomes, which is usually a fatal mistake.
Figure 10.2  Cyclin–dependent kinase. (A) The role of a cyclin–dependent kinase (Cdk) is to phosphorylate certain target proteins using ATP as the phosphate donor. Cdk requires a cyclin partner in order to be active and to recognize proper targets. Cdk targets include proteins involved in DNA replication, chromosome condensation, spindle formation, and other crucial events of the cell cycle. (B) Cdk activity can be regulated in three ways: by availability of cyclin subunits, by phosphorylation of the Cdk subunit, and by stoichiometric binding to inhibitors (CKI = cyclin–dependent kinase inhibitor).
Yeast cell cycle: Toy model

2-variable model

X = active CDK (bound to cyclin B)
Y = active APC/Cdh1 (dephospho Cdh1)

\[
\frac{dX}{dt} = k_1 - (k'_2 + k''_2 Y)X,
\]
\[
\frac{dY}{dt} = \frac{(k'_3 + k''_3 A)(1 - Y)}{J_3 + 1 - Y} - \frac{k_4 m XY}{J_4 + Y}
\]

Assumptions:
- As soon as the cyclin is present it is bound by the CDK.
- CDK/Cyclin is mainly located in the nucleus where its activity is proportional to the mass m.

Yeast cell cycle: Toy model

\[ \frac{dX}{dt} = k_1 - (k'_2 + k''_2 Y)X, \]
\[ \frac{dY}{dt} = \frac{(k'_3 + k''_3 A)(1 - Y)}{J_3 + 1 - Y} - \frac{k_4 m X Y}{J_4 + Y} \]

X nullcline: \( X = \frac{\beta}{J_2 + Y} \),

Y nullcline: \( X = p \frac{(1 - Y)(J_4 + Y)}{Y(J_3 + 1 - Y)} \),

\[ \beta = \frac{k_1}{k'_2} \quad J_2 = \frac{k'_2}{k''_2} \]
\[ p = \frac{(k'_3 + k''_3 A)}{(k_4 \cdot m)} \]

(Chap 10 from Comput Cell Biol)
In this toy model, the irreversible transitions of the cell cycle (Start and Finish) are the abrupt jumps of the hysteresis loop at the saddle-node bifurcation points. The G1 -> S-G2-M transition is driven by cell growth (though the mass m), and the reverse transition is driven by chromosome alignment on the mitotic spindle (through the activator A).

To fill out the picture in the previous section, we must identify the activator of Cdh1/APC and describe why A increases abruptly at the metaphase-anaphase transition and decreases in G1 phase.

(Chap 10 from Comput Cell Biol)
Extended model

A = Cdc14 (phosphatase)

Tyson & Novak (2002)
Yeast cell cycle: Toy model (extended)

Cdc14 is activated indirectly by a complex pathway involving Cdc20/APC, which destroys an inhibitor of Cdc14.

Cdc20/APC is itself turned on by the CDK/Cyclin complex.
For the sake of simplicity, in the model, we assume that Cdc14 (A) is directly activated by CDK/Cyclin (X).
**Yeast cell cycle: Toy model (extended)**

Equation for Cdc14 (A):

\[
\frac{dA}{dt} = k'_5 + k''_5 \left( \frac{(mX)^n}{J^n} + (mX)^n \right) - k_6A
\]

Equation for the mass (m):

\[
\frac{dm}{dt} = \mu m \left( 1 - \frac{m}{m_*} \right)
\]

logistic growth
Figure 10.5  Bifurcation diagram for (10.1)–(10.3). Asymptotic states of $X$ are plotted as functions of cell mass $m$. All other parameter values as specified in Table 10.1. Solid lines: stable steady states; dashed lines: unstable steady states; filled circles: stable limit cycles (maximum and minimum values); open circles: unstable limit cycles. (Notice that the bifurcation parameter $p$ in the previous figure is inversely proportional to $m$, so the hysteresis loop is flipped around.) There is a Hopf bifurcation on the upper branch of steady states (at $m \approx 0.57$), from which arises a branch of unstable limit cycles that disappear at an infinite-period saddle-loop bifurcation (at $m \approx 0.47$). A second branch of limit cycles at higher $m$ is stable. This branch originates at an infinite-period saddle-node-loop bifurcation (at $m \approx 0.79$) and terminates at another infinite-period bifurcation (at $m \approx 2.35$, not shown).
Yeast cell cycle: Toy model (extended)

**Figure 10.6** Simulation of (10.1)–(10.4), with parameter values in Table 10.1. (A) Mass ($m$, scale on right) and [Cyclin/Cdk] ($X$, scale on left). Cell division occurs when $X$ crosses 0.1 from above. (B) [Cdc20] ($A$, solid line) and [Cdh1/APC] ($Y$, dashed line).

- $X_{\text{threshold}} = 0.1$
- Cell division ($m \rightarrow m/2$)
Yeast cell cycle: Serious model

Towards a more detailed ("serious") model

Figure 10.7 The basic cell cycle engine in eukaryotic cells. The generic components in this mechanism correspond to specific gene products in well-studied organisms (see Table 10.2). Dynamical properties of this mechanism are determined by the set of kinetic equations given in system (10.5). A basal set of parameter values, suitable for yeast cells, is given in Table 10.1. Notice that we have given Cdc20 some ability to degrade cyclin B. This well-known interaction enforces the negative feedback loop at exit from mitosis: CycB activates IE, which activates Cdc20, which degrades CycB directly, as well as activating Cdh1.

Tyson & Novak (2002)
Yeast cell cycle: Serious model

Towards a more detailed ("serious") model

Additional cell cycle regulators:

**CKI** = CDK inhibitor (= Sic1).
- It forms a (inactive) trimer complex with CDK/CycB.
- CKI phosphorylation labels the protein for the degradation.
- CKI can be phosphorylated by CDK/Cyclin complexes.

**SK** = starter kinase ( = dimer of CDK and Cln2).
- It helps CDK/CycB to overcome its "ennemies".
- It is activated by the binding of its Cylin (whose the synthesis is under the control of some transcription factor TF).

**IE** = intermediary enzymes (represents the signal generated by the mitotic process itself).
- It activates Cdc20.
- In contrast, the "MAD pathway" inactivates Cdc20.

Tyson & Novak (2002)
Towards a more detailed ("serious") model

\[
\frac{dm}{dt} = \mu m \left( 1 - \frac{m}{m^*} \right),
\]

\[
\frac{d[CycB]_T}{dt} = k_1 - (k'_2 + k''_2[Cdh1] + k''_I[Cdc20]_A)[CycB]_T,
\]

\[
\frac{d[Cdh1]}{dt} = \frac{(k'_3 + k'_4[Cdc20]_A)(1 - [Cdh1]) - (k_4 m[CycB] + k'_4[SK])[Cdh1]}{J_3 + 1 - [Cdh1]},
\]

\[
\frac{d[Cdc20]_T}{dt} = k_5 + k'_5 \frac{(m[CycB])^n}{J_5 + (m[CycB])^n} - k_6[Cdc20]_T,
\]

\[
\frac{d[Cdc20]_A}{dt} = \frac{k_7[IEP](Cdc20)_T - [Cdc20]_A - k_8[Mad][Cdc20]_A - k_6[Cdc20]_A}{J_7 + [Cdc20]_T - [Cdc20]_A - k_6[Cdc20]_A},
\]

\[
\frac{d[IEP]}{dt} = k_{9m}[CycB](1 - [IEP]) - k_{10}[IEP],
\]

\[
\frac{d[CKI]_T}{dt} = k_{11} - (k'_{12} + k''_{12}[SK] + k''_{12} m[CycB])[CKI]_T,
\]

\[
\frac{d[SK]}{dt} = k_1 + k''_{13}[TF] - k_{14}[SK],
\]

\[
\frac{d[TF]}{dt} = \frac{(k'_{15} m + k''_{15}[SK])(1 - [TF]) - (k'_{16} + k''_{16} m[CycB])[TF]}{J_{15} + 1 - [TF]} - \frac{k'_{16} + k''_{16} m[CycB][TF]}{J_{16} + [TF]},
\]

(10.5)

where \([CycB] = [CycB]_T - [Trimer]\). We have assumed that CKI/CycB/Cdk trimers are always in equilibrium with CKI monomers and CycB/Cdk dimers: \([Trimer] = K_{eq}[CycB][CKI] = K_{eq} \cdot \{[CycB]_T - [Trimer]\} \cdot ([CKI]_T - [Trimer])\), or

\[
[Trimer] = \frac{2[CycB]_T[CKI]_T}{\Sigma + \sqrt{\Sigma^2 - 4[CycB]_T[CKI]_T}},
\]

(10.6)

where \(\Sigma = [CycB]_T + [CKI]_T + K_{eq}^{-1}\).

Tyson & Novak (2002)
Yeast cell cycle: Serious model

Simulation of the wild-type (WT) cell

The cell starts in G1:
- high Cdh1
- high CKI
- CDK/CycB low (because of Cdh1) and inactive (because of CKI)

Then, during cell growth:
- SK increases because the TF for its cyclin component its activated by the mass.
- As SK increases, CKI becomes phosphorylated and degraded.
- When CKI drops below CDK/CycB, then the active fraction of CDK/CycB starts to phosphorylate CKI, accelerating its decrease and to inactivate (through phosphorylation) Cdh20.
- Once inactivated, Cdh20 does not degrade CycB, and CDK/CycB can be fully activated.

S/G2/M: Active CDK/CycB initiates DNA replication (S phase) and, at the same time, SK initiates a new bud.

M phase:
- Meanwhile, CDK/CycB turns off TF and SK drops.
- But CycB/Cdk remains sufficiently active to keep its enemies, CKI and Cdh1 low.
- As CycB/Cdk drives the cell into mitosis, Cdc20 is synthesized (in its inactive form) and IE is phosphorylated.
- When IEP/Mad > $K_{th} = k_9 + k_T$), Cdc20 is abruptly activated. Cdc20 "tips the scales" in favor of Cdh1; CycB is destroyed, and the cell exits mitosis.
- When CDK/CycB drops below 0.1, the cell divides (m -> m/2) and the process starts over again.
Mutant cells lacking SK (\(k_\text{13}'' = 0\)) blocked in G1 with high level of CKI, active APC, and low level of CycB/Cdk

This is the phenotype of cells that lack Cln-cyclin (Cln1, Cln2, Cln3).

Mutant cells lacking SK and CKI (\(k_\text{11} = k_\text{13}'' = 0\)) are viable.

This is the phenotype of the quadruple mutant (Cln1, Cln2, Cln3, Sic1=CKI).
Integrative Analysis of Cell Cycle Control in Budding Yeast

Katherine C. Chen,*† Laurence Calzone,* Attila Csikasz-Nagy,‡ Frederick R. Cross,§ Bela Novak,‡ and John J. Tyson*†

The adaptive responses of a living cell to internal and external signals are controlled by networks of proteins whose interactions are so complex that the functional integration of the network cannot be comprehended by intuitive reasoning alone. Mathematical modeling, based on biochemical rate equations, provides a rigorous and reliable tool for unraveling the complexities of molecular regulatory networks. The budding yeast cell cycle is a challenging test case for this approach, because the control system is known in exquisite detail and its function is constrained by the phenotypic properties of >100 genetically engineered strains. We show that a mathematical model built on a consensus picture of this control system is largely successful in explaining the phenotypes of mutants described so far. A few inconsistencies between the model and experiments indicate aspects of the mechanism that require revision. In addition, the model allows one to frame and critique hypotheses about how the division cycle is regulated in wild-type and mutant cells, to predict the phenotypes of new mutant combinations, and to estimate the effective values of biochemical rate constants that are difficult to measure directly in vivo.
Integrative Analysis of Cell Cycle Control in Budding Yeast

Katherine C. Chen, Laurence Calzone, Attila Csikasz-Nagy, Frederick R. Cross, Bela Novak, and John J. Tyson
Figure 2. Wild-type cell cycle. Numerical solution of the differential equations in Table 1, for the parameter values in Table 2. The MDT for an asynchronous culture is 90 min. We show the cycle of a daughter cell (cycle time, 101 min; duration of G1, 36 min). The cycle time for a mother cell (not shown) is 80 min. Division is slightly asymmetric (daughter size at birth = 0.46× mother size at division). During G1 phase, Cdh1 is active and there are abundant CKIs. The G1→S transition is driven by accumulation of Cln2. The M→G1 transition is driven by activation of Cdc20. In panel 4, the left ordinate refers to [Cln2] and the right ordinate to [Cdc20] and [Cdc14].
Figure 4 (facing page). Mutations that interfere with the start and finish transitions. (A) Deletion of all three CLN genes arrests cells in G1 because the start-facilitators are missing. (B and C) The triple-cln mutant is rescued by further deletion of SIC1, but not by deletion of CDH1. (D–F) CDC20 mutations (deletion or temperaturesensitive lethal) block cells in metaphase (simulation not shown) can be rescued by deleting both PDS1 and CLB5, but not by deleting either gene alone.
Yeast cell cycle & bistability: Discussion

Spontaneous Limit Cycle or Hysteresis Loop?

- The cell cycle is a periodic process and can be seen as a limit cycle oscillator, as exemplified by the models of the egg embryo. By contrast, the model for the budding yeast emphasized a different picture: the cell cycle is seen as an alternation between two self-maintaining states, G1 (unreplicated DNA) and S-G2-M (DNA replication and mitosis). From a dynamical point of view, these two self-maintaining states are stable steady states of the kinetic equations describing the production of cyclin/Cdk activity and its destruction by cyclin proteolysis and CKI accumulation. The control system is bistable because of the fundamental antagonism between Cdk and its "enemies".

- Bistability is observed only within a restricted region of parameter space. The control system can thus be driven from one state to the other by parameter changes that carry the system across saddle-node bifurcation points. Because the stable state initially occupied by the cell (G1) is lost at the saddle-node bifurcation, the cell is forced to make an irreversible transition (Start) to the other stable state (S-G2-M). The opposite transition (Finish) can be induced only by parameter changes that carry the system across a different boundary, where the S-G2-M state is lost and the system jumps irreversibly to G1. These parameter changes and state transitions create a "hysteresis loop".

- The parameter changes that drive cells through Start and Finish are carried out by additional components of the control system, called "helper" molecules. Helper activity is only transient: It rises to induce a transition, but then falls back down in preparation for the reverse transition. Mutations that interfere with the rise and fall of helper proteins are usually inviable or severely compromised in progress through the cell cycle.

Remark: If production and destruction of the helpers are included in the "system" (ODEs), the system spontaneously oscillates. In that case, don't we retrieve the notion of a limit cycle? Indeed, it is a stable periodic solution, but it lacks some properties that we usually associate with limit cycles. Our intuition about limit cycles has been honed on continuous, autonomous ODEs, but system is discontinuous and nonautonomous, with \( m = m(t) \) and the prescription that \( m \to m/2 \) at division. It is thus more profitable to think of \( m \) not as a dynamical variable, but as an external parameter that drives the control network back and forth between regions of stable steady states.

Tyson & Novak (2002)
Molecular Noise

Table 1. Numbers of molecules (per haploid yeast cell) and half-lives for several cell cycle components.

<table>
<thead>
<tr>
<th>Cell cycle Gene</th>
<th># molecules per cell</th>
<th>Half-life (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>mRNA</td>
</tr>
<tr>
<td><strong>CDC28</strong></td>
<td>6700</td>
<td>2.2</td>
</tr>
<tr>
<td><strong>CLN2</strong></td>
<td>1300</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>CLB2</strong></td>
<td>340</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>CLB5</strong></td>
<td>520</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>SWI5</strong></td>
<td>690</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>MCM1</strong></td>
<td>9000</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>SIC1</strong></td>
<td>770</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>CDC14</strong></td>
<td>8500</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Budding Yeast Cells Vol = 40 fL
Yeast cell cycle & molecular noise

Molecular Noise

| Cycle Time | Mother | 87 min ± 14% 112 min ± 22% |
| Size @ Div | 68 fL ± 19% |

G1 Duration
Mean = 16 min
CV = 48%

S/G2/M Duration
Mean = 74 min
CV = 19%

Deterministic simulation

- The model consists of 58 species, 176 reactions and 68 parameters.
- Mass-action kinetics for all reactions.
- At division daughter cells get 40% of total volume and mothers get 60%
Stochastic simulation

- The model consists of 58 species, 176 reactions and 68 parameters.
- Mass-action kinetics for all reactions.
- Protein populations: ~1000’s of molecules per gene product.
- mRNA populations: ~10 molecules per gene transcript.
- mRNA half-lives: ~ 2 min.
- Reactions are simulated using Gillespie’s SSA

To obtain a good quantitative agreement with the experimental data, some kinetics parameters must be appropriately tuned.

In particular, the stochastic simulations predict that to cope with the low number of mRNA molecules, the turnover rate of mRNA should be relatively fast (i.e. short half-life).
**Stochastic simulation**


<table>
<thead>
<tr>
<th></th>
<th>Mother</th>
<th>Daughter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle Time (min)</td>
<td>87 ± 14%</td>
<td>112 ± 22%</td>
</tr>
<tr>
<td></td>
<td>89 ± 20%</td>
<td>114 ± 22%</td>
</tr>
<tr>
<td>G1 duration (min)</td>
<td>16 ± 50%</td>
<td>37 ± 50%</td>
</tr>
<tr>
<td></td>
<td>21 ± 48%</td>
<td>41 ± 48%</td>
</tr>
<tr>
<td>Size@birth (fL)</td>
<td>40 ± 18%</td>
<td>28 ± 20%</td>
</tr>
<tr>
<td></td>
<td>41 ± 23%</td>
<td>28 ± 23%</td>
</tr>
</tbody>
</table>
Stochastic simulation

Cell cycle control in eukaryotes can be framed as a dynamical system that gives a coherent and accurate account of the basic physiological properties of proliferating cells.

The control system seems to be operating at the very limits permitted by molecular fluctuations in yeast-sized cells.

A realistic stochastic model is perfectly consistent with detailed quantitative measurements of cell cycle variability.
Yeast cell cycle: References

Modeling the yeast cell cycle (by Novak & Tyson):