Outline

Cell growth as the driver for cell cycle (in microbes): coordination of growth and division

A basic principle organizing cell cycle control: why cyclin-dependent kinase activity must oscillate

Very messy regulatory machinery that provides the oscillations: two potentially independent oscillatory mechanisms working in tandem

Chemical-kinetic modeling (in collaboration with John Tyson)

Coordination of growth and division:

Hartwell and Unger 1977:

The minimum time for the cell division cycle is significantly less than the mass doubling time.

Note: mass increase (ribosomes, etc) is approximately exponential with increasing cell size. Thus the ‘mass doubling time’.

Growth and division are entrained because small cells are significantly less likely to initiate the cell division cycle than larger cells.
**Coordination of growth and division:**

Smaller cells are less likely to initiate the cell division cycle; the setpoint for the minimum size can be modulated by Cln3 (initiator cyclin) levels.

The mechanism remains unsolved.

Do cells measure nuclear/cytoplasmic ratios?

Is nuclear Cln3 a part of this measurement ratio?
A good idea:

Make a biochemical oscillator, so that some enzyme activity $X$ goes up and down.

Set up each bit of cell cycle machinery as two-step processes.

Then, connect up the cell division cycle machinery in the following way:

Step A $\xrightarrow{X} \xleftarrow{}$ Step B
One part of regulation of origin loading by Cdk activity: removing Mcm proteins from the nucleus.

The Mcm complex is loaded at origins before firing. It is essential for origin function. Cdk phosphorylation of the Mcm complex results in its exclusion from the nucleus.

See the movie:

Note nuclear fluorescence only right after cell division.

(Subtle point: fluorescence lasts a bit longer in daughter nuclei than in mother nuclei)

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Step A: assemble spindle

Step B: disassemble spindle

B-type cyclin: the oscillating component

Watch another movie!
Cell Cycle

Other cyclins

B-type cyclin: the oscillating component

Clb-Cdk1

Step A: Produce bud

Step B: Split off bud (cytokinesis)

Clb activity
A complication: 9 cyclins!

Cell division cycle

Cln3

Cln1,2

Cln5

Cln6

Cln1,2

Cln3

Clb3,4

Clb5,6

Clb1,2

'The purpose of a Rube Goldberg Machine is to build the most complicated machine possible to perform a simple everyday task.'
Oscillator components: cyclins

Cyclins work by activating a kinase.

Inactive kinase

Cyclin

Active kinase

Cyclin

Cyclin

Cdh1, Cdc20 : APC activators

Cell growth

Cell cycle

Origin loading

Origin use (DNA replication)

Spindle assembly

Anaphase

Telophase

Clb1-6

Cdh1

Sic1

Cdc20

Pds1

Cln1-3

Cdh1, Cdc20 : APC activators
The kinase phosphorylates target proteins, and thereby regulates target activity.

**Oscillator components: APC**

The anaphase-promoting complex (APC) ubiquitin ligase complex
Cdc20 and Cdh1 are adapters that bring destruction-box-containing target proteins to the APC ubiquitin ligase complex.

APC-Cdc20 activity is intrinsically LOW
Cell Cycle

Clb-Cdc28 ACTIVATES APC-Cdc20

Clb-Cdc28

APC-Cdh1 is intrinsically HIGH
Cell Cycle

Clb-Cdc28

Cdh1

P

db

Target

APC

Clb-Cdc28 INACTIVATES APC-Cdh1

Replication, mitotic entry

Cyclin

Replicate

Cyclin

APC

Origin loading, mitotic exit

Segregate
Circuitry for two different types of oscillators coexists in the wild-type budding yeast system.

Clb2 is the main mitotic cyclin.
Removing the Clb2 destruction box prevents Clb2 degradation

Removing the Clb2 destruction box (exact endogenous gene replacement) blocks cells in late mitosis
Phosphorylation of APC subunits Cdc16, Cdc23, Cdc27 by Clb2 kinase may provide an essential component for the negative feedback oscillator.

A tool: the APC-A mutant, in which the APC subunits cannot be phosphorylated due to S/T→A mutations.
Cell Cycle

APC-A:
Unphosphorylatable APC subunits. Negative feedback oscillator disabled.

X

Relaxation oscillator disabled.

Tetrad analysis

APC-A cdh1
Cannot complete mitosis

YEPGal
GAL-SIC1 ON

YEPD
GAL-SIC1 OFF

CDC16-A CDC23-A CDC27-A cdh1Δ

ura3::URA3::GAL-SIC1

SIC1 or CDC20 overexpression can restore cdh1 APC-A viability

Dr. Fred Cross, Rockefeller (KITP Bio Networks 3/26/2003)
Disabling both oscillators blocks the cell cycle; either one alone may be sufficient.

- **S/M**
  - Cdc20
  - Clb2

- **G1**
  - Cdh1/Sic1

**Rate equations**
\[
\begin{align*}
\frac{d\text{Clb2}}{dt} &= k_{\text{synth}} - k_{\text{deg}} \cdot \text{Clb2} \cdot (\text{Cdh1} + \text{Cdc20}) \\
\frac{d\text{Cdc20}}{dt} &= k_{a} \cdot \text{Clb2} \cdot (1 - \text{Cdc20}) - k_{d} \cdot \text{Cdc20} \\
\frac{d\text{Cdh1}}{dt} &= k_{a} \cdot (1 - \text{Cdh1}) - k_{i} \cdot \text{Clb2} \cdot \text{Cdh1}
\end{align*}
\]

**Genetic/biochemical tests**; revise rate equations and/or conceptual model.

**Conceptual model**

**Computer simulations** → predictions
Realistic computational analysis* shows that a double-oscillator system can account for cell cycle regulation, in wild-type and in many mutant situations.

* K. Chen, J. Tyson

Dr. Fred Cross, Rockefeller (KITP Bio Networks 3/26/2003)
The wild-type model is reasonably robust; some 'viable' mutants are very fragile.
Cell Cycle

**APC-A cdh1**

**APC-A cdh1 + multi-copy SIC1**

**APC-A cdh1 + GAL-CDC20**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect of change</th>
<th>Change</th>
</tr>
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<tbody>
<tr>
<td>kdb2p</td>
<td>Increase Cdc20-dependent Clb2 degradation</td>
<td>Up 10X</td>
</tr>
<tr>
<td>ka20'</td>
<td>Increase constitutive <em>CDC20</em> transcription</td>
<td>Up 20X</td>
</tr>
<tr>
<td>ks20''</td>
<td>Increase Clb2-regulated <em>CDC20</em> transcription</td>
<td>Up 4X</td>
</tr>
<tr>
<td>kd20</td>
<td>Decrease Cdc20 degradation</td>
<td>Down 8X</td>
</tr>
<tr>
<td>ka20''</td>
<td>Increase Phospho-APC-independent Cdc20 activity</td>
<td>Up 7X</td>
</tr>
<tr>
<td>ksc1'</td>
<td>Increase constitutive <em>SIC1</em> transcription</td>
<td>Up 8X</td>
</tr>
<tr>
<td>ksc1''</td>
<td>Increase Swi5-regulated <em>SIC1</em> transcription</td>
<td>Up 90X</td>
</tr>
<tr>
<td>kaswi</td>
<td>Increase Swi5-regulated <em>SIC1</em> transcription</td>
<td>Up 10X</td>
</tr>
<tr>
<td>Jiswi</td>
<td>Increase Swi5-regulated <em>SIC1</em> transcription</td>
<td>Up 90X</td>
</tr>
</tbody>
</table>

*cdbh1* APC-A model: test systematic parameter variations for ‘suppression’ of ‘inviability’
Why would cells have two functional oscillators?  
Maybe they function at different times...
Cell Cycle

Cyclin

Cdc20

Sic1/Cdh1: for maintaining G1?

Cld/Cdc20: for running a minimal cell cycle?

Sic1/Cdh1

Clb

Cdc20

G1

G1

G1

Frog

p27/p21/Cdh1

G1

G1/S

CycB

Cdc20

CycE, CycA

G2/M

Frog

Dr. Fred Cross, Rockefeller (KITP Bio Networks 3/26/2003)
Cell Cycle

Low nutrients, inhibitory extracellular factors

Inhibitory extracellular factors
Ralph Wäsch (CLB2-db)

Vincent Archambault (model testing)

Jamie Bean (movies)

John Tyson, Kathy Chen (Virginia Tech)