What is similar between cells and washing machines!
CELLS COME FROM OTHER CELLS

- Questions:
  - How are they duplicated?
  - How are they partitioned?
  - How are these processes coordinated?
  - How are mistakes overlooked / rectified?
Interesting facts

- 25,000,000 cells divide per minute in our bodies
- Every 8 seconds DNA suffers a break at least once!
- Some cells continuously divide (skin)
- Some cell types are quiescent, unless provoked (blood)
- Some cells never divide (muscle, neurons)
- Some cells are dead but still part of our body (hair, nails)

- Division leads to death, eventually!
- Control of cell division is control of cancer.
- Our goal is to understand cell cycle controls and discover new ones
The cell cycle is an ordered series of events leading to the replication of cells.
• Typically human cell takes 24 h to divide,
• $G_1=9h \rightarrow S \ 10h \rightarrow G_2 \ 4.5h \rightarrow M \ 0.5h$
• Cells stay in $G_1$ till growth factors, size factors signal progression
• A prolonged stay in $G_1$ is called $G_0$ (example)
• $M$ phase - $P+M+A+T$
The stages of mitosis and cytokinesis in an animal cell

(a) Interphase (G2)  (b) Early prophase  (c) Late prophase  (d) Metaphase
- Centrosomes
- Spindle poles
- Kinetochore
- Sister chromatids

(e) Anaphase  (f) Telophase  (g) Interphase (G1)
- Cleavage furrow
Centrosome duplication precedes and is required for mitosis
The mitotic apparatus is a machine for separating chromosomes.
The mitotic apparatus in a metaphase mammalian cell

Note 3 types of microtubules
Capture of chromosomes is also important for spindle assembly.
Dynamic instability of microtubules increases during mitosis
Current concept:  Bipolar spindle determines cell division plane

New concept:  Cell Division is independent of spindle polarity

Pawan Dhar. The Cell equator - more than poles apart. Trends In Biotechnology (April 2004 issue)
Major concerns:

1. ordered process - activate cellular machines only at correct time
2. Ensure completion before progression
   - replication before mitosis
   - mitosis before cytokinesis
   - growth before replication
3. Must be subject to external controls e.g, divide only when more cells needed
The original cyclin based model of cell cycle
The Pioneers


“We need to develop simplifying, higher level models and find general principles that will allow us to grasp and manipulate the function of biochemical networks”
Figure 3. Time-lapse photomicroscopy of a cdc mutant cells growing at the permissive temperature (A) and several hours after a shift to the restrictive temperature (B).

Hartwell - Nobel lecture 2001

Cdc - Cell division cycle
Checkpoints in cell cycle regulation
What is a cell cycle?
G1-S-G2-M
S <=> M (cohesin)

Q: How to ensure the orderly progression of events - nuclear cycle vs. cytoplasmic cycle?

Cell cycle is also a chromosomal cycle
Evidence for Cell cycle regulation

**Key experiment 1**

**Rao and Johnston**
S+G1: S phase starts in G1 nuclei
S+G2: No S phase in G2 nuclei
G1+G2: no S or M phase
M phase + Interphase: Mitosis

**Conclusions**
1. Diffusible factors promote S or M phase
2. S phase promoting factor only works in G1 nuclei.
3. M phase promoter works on everything
Key experiment 2

Xenopus extracts (Tim Hunt)
Group 1: Oocytes blocked at M phase
Group 2: Oocytes at post M phase

Q: Is it possible to induce #1 to enter M phase
A: Yes.

MPF was discovered!
Key experiment 3
Material: Sea Urchin Embryos, synchronously dividing
Key observation: Of all the proteins, some regularly go up and some go down with the cell cycle

Cyclins were discovered

Key experiment 4
Material: Yeast cells
Q: Is it possible to isolate yeast mutants that can grow but cannot divide their nuclei. Use these mutant combinations to identify cell cycle controls?
Breakthroughs in genetics

Why yeast? - Budding, fission yeast

Task: Look for conditional mutants

Q: How to determine the stage at which the cell is blocked?

Key finding: cdc mutants discovered (cdc = cell division cycle)

Task: Remove a key component

Q: Does it affect the pattern / timing of regulation?
**Model**

Slow down fission yeast cycle - long cells  
Speed up fission yeast cycle - short cells

Task: Isolate short or “wee” mutants  
< defective in timing or regulation >

**Q:** How many genes are mutated i.e., for all the mutants that arrest cells in G2, how many different genes do they represent ?

Task: Combine mutants together.  
**Q:** Is it possible to determine the pathways between them ?
# Case study: Fission yeast

<table>
<thead>
<tr>
<th>Mutants</th>
<th>Phenotype</th>
<th>Mutants</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>cdc25</td>
<td>long</td>
<td>cdc2-L wee1</td>
<td>long</td>
</tr>
<tr>
<td>wee1</td>
<td>short</td>
<td>cdc2-L OP cdc25</td>
<td>long</td>
</tr>
<tr>
<td>cdc2-L</td>
<td>long</td>
<td>cdc2-w cdc25</td>
<td>short</td>
</tr>
<tr>
<td>cdc2-w</td>
<td>short</td>
<td>cdc2-w OP wee1</td>
<td>short</td>
</tr>
<tr>
<td>OP cdc25</td>
<td>short</td>
<td>cdc25 wee1</td>
<td>normal</td>
</tr>
<tr>
<td>OP wee1</td>
<td>long</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cdc13</td>
<td>long</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Conclusion
1. Wee1 cdc25 act upstream of cdc2
2. Wee1 inhibits cdc2 (absence of wee1 speeds cell cycle)
3. cdc25 activates cdc2, because if cdc25 is missing, things slow.
4. cdc25 and wee1 antagonize each other, because together everything looks okay

## Model

```
G2  Protein kinase  Phosphatase
    wee1             cdc25

cdc2  +  cdc13  cyclin  M
```
Budding yeast

Budding: Cdc28/Cln1 and Cdc28/cln2
START: Cdc28/Cln3
Mitosis: Cdc28/Clb1, Cdc28/Clb2
DNA replication: Cdc28/Clb5, Cdc28/Clb6

How are CDK/Cyclin dimers regulated?
How is the transition accomplished?
1. Phosphorylation / Dephosphorylation
2. Inhibition (to regulate kinase activity)
3. Destruction - of cyclin and inhibitors (sequential and temporal)

Regulated destruction
1. Degradation of cyclins
2. CKIs e.g, Sic1, Rum1
3. Cohesin - molecular glue
Complex regulatory process
Initial Conditions (G1 phase)

During the G1 phase, Cdc20 is low in concentration, while Hct1 concentration is high. The active Hct1 ensures that the cyclins Clb5, Clb2 and Cln2 are at low concentration. Sic1 concentration is high. Cdc14 is sequestered into the nucleolus. Swi5 is active while Mcm1, SBF, MBF and MAD remain inactive. Cdc28 and APC are always in excess, available for binding to the cyclins and to Cdc20 or Hct1 respectively. APC and Sic1 are mostly in the non-phosphorylated state during the G1 phase.

The G1/S Transition (START)

The concentration of Cln3 and Bck1 is correlated to the cell size by an incompletely understood mechanism. The increased concentration of these entities triggers the conversion of SBF and MBF from their inactive form to an active form.

SBF and MBF are transcription factors for Clb5 and Cln2 respectively. As Clb5 and Cln2 synthesis rate increases, they bind with Cdc28. As the Cdc28/Clb5 and Cdc28/Cln2 complexes promote conversion of SBF and MBF to active form, they form a positive feedback loop and increase their transcription rates even further.

The non-phosphorylated Sic1 present in high concentration binds to and inhibits Cdc28/Clb5 complex. The free Cdc28/Clb5 complex triggers DNA synthesis, resulting in the cell being termed to be in the S phase. The Cdc28/Cln2 complex triggers the formation of the bud.

Clb2 synthesis remains at a basal level throughout this process. Most of the Clb2 produced is degraded by active APC/Hct1, and causes the concentration of Clb2 to be kept low.
The S Phase and the G2 Phase

Cdc28/Cln2 phosphorylates Sic1. The phosphorylated Sic1 is ubiquitinated by SCF/Cdc4 and degraded by S26 proteosomes. As the concentration of Sic1 thus reduces, Cdc28/Cln2 gets released from the Cdc28/Cln2/Sic1 complex.

The combined effect of Cdc28/Cln2 inactivates APC/Hct1 via phosphorylation. As Hct1 is inactivated, the concentration of Clb2 slowly increases due to the basal level of transcription. Clb2 complexes with Cdc28 forming Cdc28/Cln2; the complex activates Mcm1. Cdc28/Cln2 also promotes conversion of SBF to an inactive form, thus slowing down the transcription of Cln2. Cdc28/Cln2 also catalyzes the degradation of Cln3.

The G2/M Transition

The Cdc28/Cln2 complex activates transcription of Cdc20 via the MCM pathway. Cdc20 forms a complex with MAD in the presence of unaligned chromosomes. When the chromosomes are aligned, MAD is removed and Cdc20 is free to complex with phosphorylated APC. Cdc28/Cln2 phosphorylates APC to allow this complex formation.
<table>
<thead>
<tr>
<th>Factor</th>
<th>Role</th>
<th>Cerevisae</th>
<th>Pombe</th>
<th>Metazoans</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDK</td>
<td>cyclin dependent kinase</td>
<td>CDC28</td>
<td>Cdc2</td>
<td>Multiple CDKs: CDK1-6</td>
</tr>
<tr>
<td>G1 cyclin</td>
<td>regulatory subunit of CDK for cell cycle entry</td>
<td>CLN1,2 and 3</td>
<td>?</td>
<td>Cdk4-cyclinD</td>
</tr>
<tr>
<td>S phase cyclin</td>
<td>Regulatory subunit of CDK for S phase entry</td>
<td>CLB5, 6</td>
<td>Cig2</td>
<td>Cig2</td>
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<tr>
<td>late S phase cyclin</td>
<td>Regulatory subunit of CDK for S phase progression</td>
<td>CLB3, 4</td>
<td>?</td>
<td>Cdk2-cyclinA</td>
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<tr>
<td>M phase cyclin</td>
<td>regulatory subunit of CDK for mitosis</td>
<td>CLB1, 2</td>
<td>Cdc13</td>
<td>Cdc2</td>
</tr>
<tr>
<td>APC</td>
<td>Multi-component ubiquitin ligase required for degradation of substrates in mitosis and G1</td>
<td>Many genes</td>
<td>Many genes</td>
<td>Many genes</td>
</tr>
<tr>
<td>securin</td>
<td>An APC target, inhibits sister chromatid separation</td>
<td>PDS1</td>
<td>Cut2</td>
<td>securin</td>
</tr>
<tr>
<td>separase</td>
<td>The securin target, a protease that degrades cohesin</td>
<td>ESP1</td>
<td>cut1</td>
<td>separase</td>
</tr>
<tr>
<td>----------</td>
<td>------------------------------------------------------</td>
<td>------</td>
<td>------</td>
<td>----------</td>
</tr>
<tr>
<td>Cohesin</td>
<td>A complex of proteins that holds sister chromatids together</td>
<td>SCC1, aka MCD1 SCC3 SMC1 SMC3</td>
<td>Rad21 psc3 psm1 Psm3</td>
<td>Rad21 SCC1 SCC3 SMC1 SMC3</td>
</tr>
<tr>
<td>CKIs</td>
<td>CDK inhibitors—generally small molecules, not conserved in primary sequence</td>
<td>SIC1</td>
<td>Rum1</td>
<td>PV19 PV21 PV27</td>
</tr>
<tr>
<td>MEN</td>
<td>Mitotic exit network, regulates progression out of M phase in S. cerevisiae. Similar proteins in S. pombe regulate septation (called SIN, for septation initiation network)</td>
<td>TEM1 BUB2 BYR4 LTE1 CDC14 Net1</td>
<td>Spg1 Cdc16 Byr4 ? Clp1 ?</td>
<td>?</td>
</tr>
</tbody>
</table>
G₁ and G₂ arrest in cells with damaged DNA depends on the p53 tumor suppressor

(a) Wild-type cells

(b) p53⁻ mutant cells
Modeling cell cycle transactions
What’s good about Tyson’s model?

JTB 2001:210, 249-263
Philos.Trans.R.Soc.Lond. 1998: 353, 2063-76

• The first mathematical model of cell cycle
• Most elaborate analytical model
• Correctly predicts linkage between cell size and G1 cyclin (Cln3) gene dosage
• Prediction of cyclin and Sic1 abundance in various situation was reasonably accurate
Equation governing cyclin-dependent kinases:
1. \( \frac{d}{dt} [Cln2] = (k_{c,n2} + k_{c,n2} [SBF]) \cdot mass - k_{d,n2} [Cln2] \)
2. \( \frac{d}{dt} [Clb2]_r = (k_{c,b2} + k_{c,b2} [Mcm1]) \cdot mass - V_{d,b2} [Clb2]_r \)
3. \( V_{d,b2} = k_e b_2 (1 - [Hctl]) + k_e b_2 [Hctl] + k_e b_2 [Cdc20]_4 \)
4. \( \frac{d}{dt} [Clb5]_r = (k_{c,b5} + k_{c,b5} [MBF]) \cdot mass - V_{d,b5} [Clb5]_r \)
5. \( V_{d,b5} = k_{d,b5} + [Esp1] \cdot (k_{d,b5} + k_{d,b5} [Cdc20]_4) + k_{d,b5} [Cdc20]_4 \)
6. \( [Cln3] = k_e \cdot mass \)
7. \( [Clb2]_r = [Clb2] + [Clb2/Sic1] \)
8. \( [Clb5]_r = [Clb5] + [Clb5/Sic1] \)
9. \( [Sic1]_r = [Sic1] + [Clb2/Sic1] + [Clb5/Sic1] + [Sic1P] \)

Equation governing the inhibitor of Clb-dependent kinases:
10. \( \frac{d}{dt} [Sic1]_r = k_{c,e1} + k_{c,e1} [Swi5] - k_{d,e1} [Sic1]_r \)
11. \( \frac{d}{dt} [Sic1]_p = \frac{\gamma_{edk,sic}}{k_{m,s} + [Sic1]_r} \cdot \left( k_{p,s} [Cdc14] + k_{d,s} \cdot [Sic1]_p \right) \)
12. \( \gamma_{k_4} = k_{c,4} + k_{c,4} \cdot \frac{\gamma_{edk,sic}}{k_{m,s} + [Sic1]_r} \)
13. \( \gamma_{edk,sic} = \gamma_{c,3} \cdot \left( k_{c,ln3} + 0.1 \cdot [X] \cdot [mass] \right) + \gamma_{c,2} \cdot [Cln2] + \gamma_{c,5} \cdot [Clb2] + \gamma_{c,b2} \cdot [Clb2] \)
14. \( \gamma_{c,ln3} = \max_{c,ln3} [Cln3] \cdot \left( [Cln3] + k_{c,3} \right) \)
15. \( \frac{d}{dt} [Clb2/Sic1] = - k_{u,2} [Clb2/Sic1] + k_1 [Clb2] \cdot [Sic1] - \left( k_{d,2} + \gamma_{k_4} \right) \cdot [Clb2/Sic1] \)
16. \( \frac{d}{dt} [Clb5/Sic1] = - k_{u,3} [Clb5/Sic1] + k_1 [Clb5] \cdot [Sic1] - \left( k_{d,3} + \gamma_{k_4} \right) \cdot [Clb5/Sic1] \)
17. \( \frac{d}{dt} [Swi5]_r = k_{c,swi5} + k_{c,swi5} [Mcm1] - k_{d,swi5} [Swi5]_r \)
Work at BII

The Budding Yeast
*Saccharomyces cerevisiae*

- **Mother**
- **Daughter**

**Bud Scars**

Forward modeling
Mandar Chitre (NUS)
Meng How (NTU)

Reverse engineering
Dr. Sun Yan (BII)

Courtesy: Uttam Surana, IMCB
Our Cell cycle Model

- 37 entities - *its growing*
- 51 reactions
- 78 parameters
- 17 initial values
- Takes 30 min on a 1.6 GHz Pentium IV to run a 120 min simulation
Which experimental results do we intend to confirm?
Pattern of Destruction*

* Dr. Uttam Surana, IMCB
Cell Cycle Pathway Model in *Saccharomyces cerevisiae* by Mandar Chitre, Mar 2003

Forward modeling
SGD Expression Data

• Possible constraints:
  – Strong constraints
    • Relative concentration of Cyclins at constant time step
  – Weak constraints
    • Minimum/maximum relative concentrations of Cyclins
    • Cell cycle time
• Should constraints include non-Cyclin concentrations ?
• Which synchronization method is most similar to an undisturbed budding yeast cell ?
Training Constraints

• Automated tuning can be based on observed constraints derived from SGD data.
• However, SGD uses 4 different synchronization methods:
  – Alpha factor
  – CDC15
  – Cell size
  – CDC28
• Each method leads to different cell cycle parameters – amplitude, cycle time, etc.
Problems with numerical model

Figure 3. Expected oscillatory behavior of the key cell cycle regulated Cyclins

Mandar 2003
Multi-state logic model

Qualitative model on MEN

WILD TYPE
Viable
2: START
3: DNA Synthesis
3: Budding
6: Mitosis
7: Chromosomes aligned
9: Chromosomes segregated
11: Cytokinesis
Reverse Engineering

Dr. Sun Yan’s work

1. Wavematch method v/s Spellman method
2. Identified **NEW** genetic regulators of cell cycle from genomic expression profiles
Reading material

Dr Forsburg's all-purpose Cell Cycle Lecture Notes
http://pingu.salk.edu/~forsburg/cclecture.html

http://mips.gsf.de/
Yeast: http://mips.gsf.de/genre/proj/yeast/index.jsp